

Prejudice and academic tenure

The British Government plans to intervene directly in the ancient argument about the terms on which university teachers are appointed. It may irreparably damage the universities in the process.

BRITISH governments, even those like the present which profess that government should be unobtrusive, are chronically inclined to centralism. That is the only charitable reading of the decision (see *Nature* 17 May, p.197) that the government intends to intervene directly on the issue of academic tenure in British universities. The plan, which will no doubt be legitimized by the government's huge majority in the House of Commons, is that a group of unnamed statutory commissioners should be given the power to amend the charters which allow British universities to operate as such. The excuse, according to the Secretary of State for Education and Science, Sir Keith Joseph, is that universities have been slow, these past two years, voluntarily to extinguish academic tenure and are probably constitutionally incapable of doing so. The truth is that the present government is deeply offended by the common (but not universal) clause in British academics' contracts of employment that appointments will continue until "normal retirement age", and is impatient to see these arrangements swept away. By proposing to intervene directly, however, the government has confirmed its reputation as one that lets prejudice dictate policy.

The case for academic tenure is widely misunderstood, but derives from the doctrine that universities are self-governing communities of scholars within which people may (and will) disagree on questions as different as the allocation of car-parking space and the best route to the quantization of the gravitational field. The past six centuries have provided ample evidence that universities are most creative when individuals are able to follow lines of inquiry which their immediate colleagues may consider unproductive, and which fall outside public definitions, by governments and others, of social needs. Both in research and teaching, the indispensable social function of universities is the level-headed iconoclasm of which they are the only continuing source. Unfortunately for the government, this requires that academics should enjoy a measure of intellectual independence even from those who are titularly superiors. It would be (and when attempted is) disastrous if heads of academic departments sought to organize their bailiwicks' research as if it were an industrial research programme directed at a well-defined objective. In industry, it is proper that somebody unwilling to conform with the overall plan should be asked to find a job elsewhere. Human nature being what it is, heads of university departments are not always free from the temptation to ponder the advantages of such arrangements. The freedom to dissent even from close colleagues' opinions is what the tenure system was intended to preserve.

Security

But why should academics be insulated from the market forces to which all other people are exposed? That is what the British Government has been asking these past five years. The question is plausible enough, but begs another provoked by the way in which the British Government itself has been rigging the market in which British universities now operate. The decree more than three years ago that student numbers must be limited, and the foolish translation of this demand into student quotas for every British university by the University Grants Committee, means that British academics are unable to strive to ensure their own security by better satisfying the demand for higher education. Special

public sponsorship, more recently, for the provision of higher education in fields such as information technology, while generally welcomed by hard-pressed universities, is another sign that the academic market is not a genuine interaction between self-governing communities of academics and would-be students but a process in which often arbitrary external influences play an important part. But unpredictable changes in the environment in which academics must work are not always as remote. Last week's report from the science board of the Science and Engineering Research Council (see p.297), the chief source of financial support for "small science" in British universities, says that, in future, industrial applicability will be one of the chief criteria for deciding how research grants will be awarded, thus loading the dice against academic scientists whose interests lie elsewhere. When fashion and prejudiced opinion have such an important influence on the academic market, it would not merely be unfair to expect that academics should allow their careers to be determined so arbitrarily, but would probably be impossible: potential academics would prefer to look for jobs elsewhere.

Implications

None of this implies that the tenure system as it is is sacrosanct. On the contrary, there is both a need for change and evidence that universities and those who work in them agree. Plainly, if a university as such should cease to exist, it would be unreasonable of its employees to expect that their employment should nevertheless continue. Similarly, if a university should find that student demand for some of the services it offers has collapsed, and those concerned are unable to discover other opportunities to exploit, it is only seemly that they should be invited to pursue their academic interests in some other environment. For while universities are, or should be, self-governing communities of scholars whose unique product is the outcome of successful research, their bread and butter, now as in the twelfth century, is the education of students. But the revision of the rules of tenure which the British Government is seeking will be equitable and tolerable only if it is accompanied by a restoration of some semblance of each university's freedom to decide for itself what to teach, and to whom.

The specific implications of this simple rule are plain. First, the student quotas, which are unfair to would-be students and a source of serious diseconomy, should be abolished. Since the system exists only because the government cannot think of any other way of limiting the aggregate amount of what it is required by law to pay to local authorities in respect of university fees and maintenance grants, it should quickly bend its ingenuity to helping young people now denied higher education to pay for it. Second, the market in higher education should be made to work freely in the sense that universities should be rewarded according to their effectiveness in satisfying the demands made on them. As things are, individual universities may find departments of which they are proud snatched away because some committee has decided that another arrangement would better meet the national interest. Finally, for restriction of tenure to be equitable and acceptable, universities must not merely be free to make academic decisions for themselves (with the risk of being wrong some of the time), but must do so in such a way that those whose careers are affected fully participate.



If these conditions could be met, the heavy-handed mechanism the British Government now plans to use for the enforced restriction of tenure (on new appointments only) would not be necessary. Moreover, some of the devices that have been suggested for amending the standard form of academic tenure — longer probationary periods or even periodic reviews — would be seen to be irrelevant, even counter-productive. For younger academics, and those whose chief contributions to the work of their universities is in teaching rather than research, are just as much in need of protection from tyrannical superiors as those whose research is off the beaten track. Thus the standard terms of tenure should be amended only so as to allow appointments to be terminated when a university is forced by financial considerations to contract or when, after serious and open discussion within an institution, a change of academic policy seems prudent. The need to demonstrate due process should be paramount.

Even at this late stage, the British Government should recognize that it is more likely to damage its university system beyond repair by forcing amendment of academic tenure through the parliamentary system than to gain from winning a symbolic fight with institutions already all too painfully aware of being in its pocket. Victory, of course, would allow the government to claim that it had put academics on the same basis as other professional people. Only with the passage of time would it become apparent that the supply of would-be academics had dried up. Indeed, it is even possible that signs of damage would never become so obvious that the government would be compelled to notice. Good people, notoriously mobile as things are, would seek posts in research institutes or universities elsewhere, allowing vacancies to be filled by the less able. Is that what the British Government wants? □

Banking under strain

The near-collapse of a US bank shows the need for a deal with developing countries.

THE unwelcome shadow of the Great Crash of the 1930s was abroad like the reaper last week, and it is too soon to know whether it has gone for the time being. Just as the First World War began with an assassination in the obscure Serbian city of Sarajevo, so the decade of economic upheaval of the 1930s began with the collapse of an obscure Austrian bank of which most of those affected had not previously heard. Last week's difficulties were inevitably better publicized. On the Monday, a group of New York banks announced that they had clubbed together to provide \$4,500 million of credit to the Continental Illinois Bank and Trust Company, a kind of bankers' bank which has so far made its way in the world by borrowing on a short-term basis from other banks and lending on a long-term basis to other kinds of institutions, mostly commercial companies. After two disastrous years, partly attributable to a rash of light-hearted lending to oil-exploration companies (through the intermediary of an Oklahoma bank which collapsed in July 1982), the bank's bad debts had grown to more than \$2,000 million, more than the value of its capital. So, inevitably, other banks became chary of lending to Continental Illinois, which is said to have been borrowing overnight money from its fellow banks to the tune of \$8,000 million each day. By this yardstick, it is hardly surprising that even the \$4,500 million was not enough to still the rumour mills, so that by the end of the week the US Federal Reserve, the lender of last resort, was forced to chip in a further \$7,500 million to prevent Continental Illinois from going bust.

Why should that matter? Why should the Federal Reserve have gone to such trouble to save an over-ambitious bank from collapse when there are many in Congress who think it high time that the banking industry, not noticeably unprosperous, was taught a lesson? And why, if it comes to that, had not the Federal Reserve diligently exercised its supervisory powers to ensure that Continental Illinois lived less hazardously? All the answers are implied by a ringing declaration put out last weekend by the principal debtor nations of Latin America — Argentina, Brazil,

Colombia and Mexico, which between them have external debts of \$240,000 million. In the past two years, each of these governments has been compelled to ask its creditors for more time to pay, and for extra help (from the International Monetary Fund), and as a consequence has had to accept deflationary domestic policies. (In Argentina, for which a rescue package has still to be arranged, the rate of inflation is now 480 per cent a year.) At the weekend, the four governments declared that they "cannot indefinitely accept" that "the aspirations of our peoples for development, the progress of democratic trends in the region and the economic security of our continent" should be put in hazard by high interest rates on borrowed funds and by the protectionism now apparent to suppliers of primary raw materials. This telling statement explains what has been happening in the United States because it draws attention not merely to the plight of the debtors but to the vulnerability of their creditors. The commercial banks could fall like dominoes if, in these delicate circumstances, one of them keeled over, while banking's supervisory agencies, accustomed as they have become to letting banks count as assets even their most dubious loans to developing countries in money trouble, plainly cannot be over-diligent in regulating the banks' domestic business.

The reasons why this underlying delicacy has so suddenly become apparent are also simple, and are to be found in Washington and New York. The administration's huge budget deficit, in round numbers £200,000 million this calendar year, has somehow to be matched by persuading people somewhere to buy US Government securities. If the United States were financially isolated, the result would be a high rate of domestic inflation. In the real world, much of the borrowed money comes from elsewhere, from overseas investors attracted by the high rate of interest the federal government must pay and also by the signs there have been, in the past year, of a return to rapid economic growth. The result of all this is that the dollar has been strong relative to other currencies and that the terms of trade have moved against the United States, which is likely this year to spend \$80,000 million more on imports than it will earn from exports (which will again be financed by borrowing from abroad). But an election year is hardly the time to expect the federal government to change its tack.

Where all this will lead is anybody's guess. To the extent that Continental Illinois will now have been able to replace some of its short-term debt, interest rates should fall temporarily but at the expense of the inflation rate (which will be slower to respond). Sooner or later, there will have to be a settlement with Continental Illinois, in the course of which the bank's shareholders will be the chief losers (the consequences of which will be modestly deflationary). Whether these mildly beneficial effects will last until the presidential election in November is uncertain; most probably, they will not, for the commercial banking system will long before then be grappling with the next crisis among the debtor nations. To judge from the weekend declaration, that negotiation will be more tricky than the successful attempts to reschedule the payments of debt which there have been in the past two years, when the commercial banks have safeguarded their assets (loans) by lending debtors part of the money required to pay past interest, relying on tough economic recipes dictated by the International Monetary Fund to help ensure that the amounts outstanding will also in due course be paid.

What the Latin-American debtors now say is that they cannot continue to toe the bankers' line. Instead, they plead that there should be a conference later in the year to consider ways in which they could earn their way out of trouble by being helped to sell raw materials to their industrialized creditors. The catch is that the volume of debt is far too great to make that a practical proposition, even though, on this occasion, the commercial banks will be eager for any help that such a device could bring. The stratagem of replacing all the debtor nations' obligations by long-term loans from some international organization, which would safeguard the commercial banking system, is in many ways objectionable, but may be the only course open to the industrialized West when it holds its summit meeting in London next month. □

Direct broadcasting satellites

Japanese fury over US component failure

Tokyo

THE failure of US-assembled components in Japan's Yuri 2A satellite — the world's first direct television broadcasting satellite (DBS) — has provoked a furious reaction from the Japanese Government. In language normally reserved for serious clashes over trade imbalances, the Minister of Posts and Telecommunications, Keiwa Okuda, told visiting US Vice-President George Bush last week that the government took a "serious view" of the breakdown of two out of three of the satellite's transponders.

Yuri 2A was launched in January to improve reception of the state broadcasting system (NHK)'s two television channels for around one million people living in mountainous regions, remote islands and high-rise "urban canyons". But with only one transponder functioning, NHK has abandoned plans to broadcast its second educational channel. A spokesman for Toshiba, the satellite's main contractor, said the malfunctioning units were manufactured by Thomson CSF of France and assembled by General Electric in the United States.

The angry reaction to the failure does not, however, reflect disappointment at the loss of educational broadcasts but the critical role Yuri plays in a whole set of conflicting aspirations for the Japanese telecommunications, satellite and electronics industries.

All the technologically-advanced nations are now making plans to introduce direct satellite broadcasting. By being first off the mark, Japanese manufacturers had hoped to establish themselves as the number one suppliers of receiving equipment. The parabolic antennas and decoders needed to receive NHK broadcasts are already on sale in every corner electronics store in Japan, and Toshiba and a Nippon Electric Company (NEC) affiliate have been named as suppliers by Satellite Television of the United States, which hopes to have its own satellites ready by the end of 1985. The failure of Yuri is bound to affect sales in the home market, which would have built up experience for the international market.

The same companies are also eyeing the much bigger technological opportunities to be offered by DBS in the future. Japanese companies believe that DBS will be used in the 1990s to begin high-definition television broadcasts. Already Japan is the world leader in this technology. As long ago as 1968, NHK research laboratories had produced a system with 1,125 lines — almost twice the number currently in use in the United States and Japan (525) and in

Europe (625) — which gives a picture no different in quality from commercial 35-mm movie projection. The trouble is that this NHK technological advance can only be capitalized upon if the 1,125-line system is adopted as an international standard. Strong competition is now coming from a 925-line system developed in the US Advanced Television Project at Massachusetts Institute of Technology. Technically the US system looks superior because it requires less transmission capacity.

Both Sony and Matsushita Electric will have 1,125-line systems ready for the marketplace by 1986. And by getting working systems out as soon as possible — beginning with mini "video theatres" — they hope to push international acceptance of the 1,125 standard. Any slowing in the pace of satellite development is seen as a long-term threat to the technological lead they will have when DBS becomes available for high-definition television.

More immediately worried by Yuri's failure are big businesses which want to buy communications satellites to transmit their own business data. Only a year ago, Keidanren (the Federation of Economic Organizations), backed by the satellite building lobby, was insisting that all Japan's satellites be domestically developed and the government was refusing

to permit businesses to import satellites. It was a combination of pressure from the United States, which saw yet another import barrier, and from businesses which want to use satellites and know that it will be far cheaper to buy them ready-made from the United States, which forced the government to change its mind at the end of last year and to allow at least communications satellites — if not other categories — to be imported.

The failure of Yuri now has the domestic satellite makers screaming for foreign imports to be stopped again — on the grounds that they are too unreliable. But the potential users have already formed a 30-company group to purchase a communications satellite from the United States. Their counter-argument is for a domestic satellite which will still have to contain a high percentage of foreign parts.

Criticism is also coming NHK's way for having the satellite built with exceptionally powerful transponders. This move had been strongly opposed by engineers favouring the use of already-tested designs but had gone ahead to make it possible for the transmissions to be received on smaller antennas. Now NHK is wondering what to do with Yuri 2B, an identical satellite scheduled for launch in the summer of next year. Should it be rebuilt or launched as it is?

For the time being anyway, NHK is cutting its losses by licensing the satellite as an "experimental" rather than "broadcasting" service with the Ministry of Posts and Telecommunications. No charges will be made for those who have bought antennas and are receiving Yuri's fitful broadcasts.

Alun Anderson

Copyright for software in Australia

Canberra

THE Australian Government has announced its immediate intention to extend to computer software the copyright protection usually given to literary works. This should end the present uncertainty about the legal status of software following

the decision of Mr Justice Beaumont in the APPLE COMPUTER *versus* COMPUTER EDGE case late last year that certain types of computer software were not protected by copyright under the 1968 act. The case is still under appeal before the full federal court.

A joint statement issued on 15 May by the Attorney-General and by the Ministers for Industry and Commerce and for Science and Technology, says that the intended inclusion of software in the existing copyright category of "literary works" rather than under the less restrictive patent law is intended to stimulate research and development in the local software industry.

Unless a federal full court decision supervenes, it is intended that passage of the proposed legislation should be secured in June, and should be understood as a short-term measure. It will not pre-empt consideration of fundamental issues remaining to be resolved, both at the domestic and international levels, on long-term policies for the protection of software and works stored in or created with the aid of computers.

Jeffrey Sellar



Genetic engineering

Rifkin wins interim injunction

Washington

AN experiment that would have been the first to release recombinant organisms to the environment was blocked last week by a federal judge (see p. 301). Granting a motion filed by anti-genetic engineering activist Jeremy Rifkin, Judge John Sirica ordered the University of California not to proceed with the experiment of Drs Steven Lindow and Nikolas Panopoulos until a full hearing can be held on Rifkin's lawsuit, which challenges last year's approval of the experiment by the Recombinant DNA Advisory Committee (RAC) of the National Institutes of Health (NIH). RAC was also enjoined from considering other proposals for deliberate release of recombinant organisms while the case is pending.

The Lindow experiment was to have tested the ability of altered strains of the bacterium *Pseudomonas syringae* to protect potato plants in the field against frost damage. The altered bacteria have a deletion for the gene responsible for an ice-nucleating protein excreted by the wild bacteria on the leaves of plants.

Attorneys for the University of California said they would immediately appeal. According to Dr Lindow, if the experiments cannot begin this week or next, they will be delayed until the autumn, when temperatures at the test site in northern California once again drop below freezing. The experiments, originally planned for last autumn, were postponed when Rifkin threatened at that time to seek an injunction (see *Nature* 306, 349; 1983).

Sirica's unusually detailed ruling on the preliminary injunction strongly suggests that he will also find in Rifkin's favour when the hearing is held. Rifkin is claiming that NIH should have filed environmental impact statements before approving the Lindow experiment. The ruling does not affect private industry, but only experiments carried out with NIH funds. Advanced Genetic Systems has submitted a proposal to RAC for an experiment virtually identical with Lindow's which is likely to be approved at the next meeting of the committee.

Although the ruling is a victory for Rifkin, Sirica made clear that he was making no judgement on the scientific issues that Rifkin had tried to raise. Sirica's judgement says that he was deciding only "narrow legal questions" and, during last week's hearing, he repeatedly rebuffed attempts by Rifkin's attorney to present testimony from scientific experts.

If Sirica finds for Rifkin in the full hearing, the immediate effect will merely be to require an environmental impact statement, but Dr William Gartland, executive director of RAC, said that process would take a year or more. The consequences might be that investigators

would meanwhile carry out their experiments in other countries — or industry-sponsored research would proliferate.

Lindow's experiment, first considered in October 1982, was unanimously approved at the April 1983 meeting of RAC. During its brief deliberation, the committee noted that Lindow had already performed identical experiments involving chemically-mutated bacteria without ill effect and that the experimental proposal had been modified to meet earlier concern.

On both occasions, the proposals were published in the *Federal Register* for public

comment; none was received on either occasion. According to Sirica's ruling, NIH made two mistakes in handling the matter. First, they failed to prepare a "programmatic" environmental impact statement in 1978, when the guidelines were modified to permit deliberate-release experiments at the NIH director's discretion.

Second, they failed to carry out even an "environmental assessment" of the Lindow experiment. Sirica rejected the US Government's argument that RAC's deliberations constitute an environmental assessment, saying he was "confident that the plaintiffs have identified several areas of plausible environmental concern which are not rebutted on the record before this Court".

Stephen Budiansky

Andrei Sakharov

Birthday gloom and celebrations

New York

LAST Monday, 21 May, the 63rd birthday of Dr Andrei Sakharov, the dissident Soviet physicist now in internal exile in Gor'kii, was marked by various and diverse events. In the Soviet Union, Dr Sakharov himself, who with his wife has been on an open-ended fast since 2 May, was removed, over the weekend, to a nursing home, where he is reportedly being forcibly fed. At the same time, the Soviet media stepped up their attacks on Sakharov's wife, Elena Bonner, on whose behalf he has undertaken the fast, accusing her, in effect, of exploiting her husband. In the United States, however, the day was marked by a concert in New York's Carnegie Hall, and the conferring *in absentia* of two honorary degrees, by the Universities of Long Island and of Pennsylvania.

The concert and the degree ceremonies were, of course, planned long ago. Nor is this the first time that Sakharov has received an honorary degree. Moreover, some of the formal speeches placed relatively little emphasis on the current plight of Sakharov and his wife, but delivered very much the same message on the scientist's responsibility to society as they would otherwise have done.

Sakharov's physicist colleagues, however have responded to the latest events. Last week, 36 members of the US National Academy of Sciences (of which Sakharov is an honorary foreign member), including 13 Nobel laureates and the presidents of the American Academy of Arts and Sciences and the American Physical Society, sent a cable to the Soviet leader, Konstantin Chernenko, stressing their "deep concern" and saying that the death of either Sakharov or his wife would create "very severe and long-lasting damage to efforts to maintain and to improve collaboration and cooperation with our Soviet colleagues". A "humanitarian act to save the Sakharovs", rather than "the creation of martyrs" is, they urge, "surely the common concern of all".

The Soviet view, however, is that they are *not* creating martyrs, but forestalling an anti-Soviet plot. Adequate treatment for Mrs Bonner's heart condition is, they say, available in the Soviet Union. Her demand to go abroad (the repeated refusals of which triggered the fast) is, according to TASS, the official Soviet news agency, only so that she might become "one of the leaders of the anti-Soviet scum on the payroll of the Western special services".

As early as 4 May, TASS described a scenario in which "with the participation of American diplomats... Sakharov would declare another 'hunger strike' while Bonner would receive 'asylum' in the US Embassy in Moscow" for the purpose of meeting foreign correspondents and "for the transmission abroad of slanderous fabrications about the Soviet Union. This 'operation', TASS claimed, was "frustrated" by the "timely measures taken by the Soviet law-enforcement agencies".

The US Embassy in Moscow and the State Department in Washington immediately denied any US involvement in such a plan. Last Saturday, however, the *New York Times* quoted unnamed State Department officials as admitting that Sakharov had appealed to the embassy to shelter his wife during the fast but that, since the embassy had not received Sakharov's letter until after Mrs Bonner had left Moscow for Gor'kii, they had not discussed either the appeal or the fast with her. The same issue of the *New York Times* also carried a story, originating from Jean Daniel, editor of the Paris weekly *Le Nouvel Observateur*, claiming that there had been "indirect" approaches from Soviet diplomats suggesting that the Sakharovs would be allowed to leave the Soviet Union if President Francois Mitterrand of France called for a halt to the deployment of medium-range nuclear missiles in Western Europe by the North Atlantic Treaty Organization (NATO).

Vera Rich

UK research support

Industrial promise to be blessed

THE science board of Britain's Science and Engineering Research Council (SERC) has given notice to the research community of how it wants to develop its new "active" research funding policy. "Clear industrial potential" seems to be one guiding principle. The board has drawn up a novel classification of the research it finances and has initiated reviews which, it claims, are already suggesting neglected areas.

In a policy statement published last week, *A strategy for support of core science*, the board says that reviews being carried out by its separate committees stress the importance of access to advanced instrumentation and adequate provision of personnel. In chemistry, where the most progress has so far been made, the synthesis of new materials and the study of catalytic reactions at surfaces will receive a higher priority, while low-dimensional structures, protein engineering and chemical sensors are among interdisciplinary areas identified as "research themes" deserving more support. For biology, image-processing and neuroscience are short-listed priority areas.

The science board is the largest of the four main arms of SERC and finances research worth £70 million a year. But it has been under increasing financial pressure in recent years, and this has emphasized the need to take a more directive role in selecting research areas, according to its chairman, Professor John Cadogan. Research grants to university workers, for example, fell from £24 million in 1977-78 to £19.5 million in 1982-83 (1982 prices), and 30 per cent of grant applications deemed worthy of "alpha" status are now going unsupported. The remainder of the science board's budget divides roughly equally between supporting research students and maintaining and developing central and regional facilities such as the synchrotron radiation source at Daresbury, Cheshire, and the central laser facility at the Rutherford-Appleton laboratory in Oxfordshire, as well as university installations. Cadogan is quite candid about his aim of securing a bigger slice of SERC's budget for the science board.

Many feel that astronomy and nuclear physics, each of which has its own board within the research council, are over-represented on SERC's governing body. A recent proposal to merge them was dropped despite the personal backing of SERC's chairman, Professor John Kingman. Cadogan, an industrial chemist, is diplomatically noncommittal, suggesting that maybe the time was not right. But the failure of the idea will not have made his crusade for core science any easier.

The new strategy is an attempt to get away from the system used to assess grant applications before 1982, when each was assessed strictly on its own merits. Now the

board will decide what the priority subjects should be, although it stresses that there will be continuing reviews.

It seems possible that in future more research with clear economic potential will be supported. Here the new strategy document appears somewhat uncertain. "It is not the science board's job or intent to support research specifically for industry", it declares boldly, adding, however, immediately afterwards that it "believes it must support core science in such a way that British industry prospers". Nevertheless, the board insists, it remains a fundamental tenet of its philosophy that funds must be made available for "outstanding scientists" on any problem of "excellence, timeliness and promise". The idea of a special fund for such "blue sky" research was dropped, however.

The science board's most urgent priority now is to increase again the amount that it can spend on research grants, and a strong case along these lines is contained in SERC's submission to the Advisory Board for the Research Councils on scientific opportunities for 1985. Something of the new subject strategy exercise has also found its way into this document, although the full impact will not be felt until next year's survey of opportunities for 1986. In the meantime, the details of "research themes" in most subjects are still being worked out, and the science board hopes its new document will be circulated widely and attract comment.

Tim Beardsley

Education in Canada

Washington

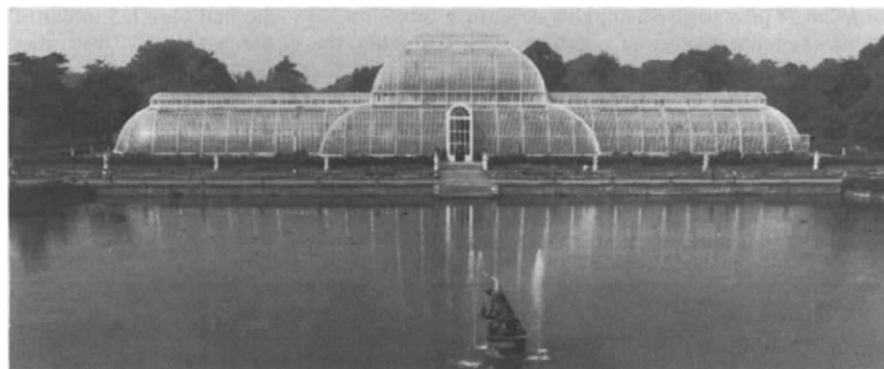
THE Science Council of Canada has joined the international stampede to declare the need for "sweeping" changes in the ways its schools teach science. In a new report for the Ministry of Science and Technology, the council says that most teachers are underqualified and that science textbooks and curricula present a picture of science so oversimplified that it is no longer authentic. Unlike a similar report published last year by the United States' National Science Board, however, the Canadian study claims that dramatic improvements can be made without large expenditure and by working through existing institutions.

The report's principal recommendation is that all elementary school children should receive science education as proposed in the official policy statements of provincial ministries of education. The Science Council concluded that these policies are seldom observed and that most children are given only "token" exposure. The council is impatient with the argument of many elementary schools that science is "integrated" in other class activities, believing "integration" to be so vague as to be of little value. It says science should be taught as a specific subject for at least 10 per cent of the time.

Another major issue raised in the Canadian report is the quality of science textbooks. The council does not quarrel with their accuracy, but says they fail to convey a realistic sense of the way in which research is conducted and of the link between science and technology.

Peter David

Kew palms off its Victorian critics



THE normally serene surroundings of the Royal Botanic Gardens at Kew, on the outskirts of London, have been rudely disturbed in recent months.

Bitter arguments have raged over plans largely to reconstruct the outstanding 19th-century palm house (above), designed by Richard Turner and Decimus Burton and the inspiration for tropical plant houses the world over.

The building is in poor structural condition and now has to be painted every two years, while 600 panes of specially curved glass have to be replaced annually. But

renovation plans put forward by the Property Services Agency of the Department of the Environment have aroused the ire of the Victorian Society, among others. The existing wrought-iron glazing bars will have to be replaced with stainless steel, because wrought iron is not now available in sufficient quantity.

Some sort of compromise on the scheme has now been reached. But the Garden History Society is still unhappy with plans to increase the extent of the plant beds and so cover the iron grating floor, although the plants grow best in beds. Tim Beardsley

Silicon manufacture

Monsanto sets up in Britain

THE United States chemicals group Monsanto last week announced plans for a £65 million investment in a new British silicon wafer research and production operation. The intention is further to improve crystal purity, which will involve developing new instrumentation, and eventually to manufacture wafers of up to 8 inch (200 mm) diameter. Monsanto expects to collaborate extensively with British university departments. World supplies of silicon wafers manufactured to the required purity and high degree of flatness are at present restricted to relatively few manufacturers. Demand is expected to increase rapidly, however. Monsanto is a leading supplier to the semiconductor industry in the United States but lags in Europe. The new Monsanto facility will be at Milton Keynes, a new development town in central England. The company explains that it was looking for a green-field site, which Milton Keynes possesses in abundance, and that Britain was chosen from a short-list of eight European countries. The British Government has helped with grant assistance, although the amount is not being made public.

In declaring its intention to move towards 200-mm wafers, Monsanto seems to be making the point that it wants to keep two steps ahead of the competition: else-

where, 150 mm is proving difficult. Monsanto boasts leadership in producing 125-mm wafers in a peak-to-valley flatness of less than 2 micrometres. But as modern wafer-stepping semiconductor manufacturing techniques become more widespread, edge-to-edge flatness will cease to be a major constraint. Local slope and crystal purity may become the limiting factors to cramming semiconductor devices onto wafers.

Two trends are combining to make purity a problem. First, chips are becoming larger, so that there is a decreased chance that each chip will be flawless. Second, channel widths are now moving down to the order of micrometres so even very small local deformities in crystal structure can disrupt a device. Implanted ions tend to spread from their original deposition sites during the manufacturing process, for which allowance has to be made in the design. The extent of the spread is determined by the structure and purity of the original lightly-doped crystal, and this is where the thrust of the research effort is likely to be. Initially, Monsanto will import wafers from the United States for cleaning and polishing in Britain, but will later embark on crystal manufacture, hoping eventually to supply 50 per cent of the British silicon market.

Tim Beardsley

US high-technology investment

Partnerships lose shine

Washington

THE downturn in high-technology stock prices is hampering efforts by biotechnology companies to raise research and development funds through other routes as well. Last week, Biogen revealed that it has raised only \$25 million of the \$40 million it had been counting on for a research and development partnership to finance the company's commercial development of gamma-interferon and interleukin-2. In recent weeks, other biotechnology research and development partnerships have met with a similar reception from investors. Hybritech was able to raise only half of the \$80 million it was aiming for; and Centecor, which had hoped to raise \$10-\$15 million, now says it will be happy with \$5 million.

Research and development partnerships are attractive to companies because they can provide stable support for long-term research and because they are a source of capital which does not dilute the equity holding of existing shareholders. Private investors obtain immediate tax benefits and later receive a share of the royalties from any inventions arising from the research. The partnerships are especially useful to emerging industries such as biotechnology which do not yet have products on the market and thus do not have a reliable source of revenue. They also give the companies far greater freedom than they would have if they entered into agreements with a single corporate research sponsor.

In the Biogen offering, the company ended up putting \$35 million of its own money into the research and development partnership, considerably more than the \$20 million it had planned.

The lack of enthusiasm for the partnerships reflects several developments. Biotechnology stocks have not been doing well on the market lately. What is more, several limited research and development partnerships in the computer field have recently received unfavourable attention for their failure to produce commercial products, which has tended to give all high-technology ventures a bad name. The most prominent case was that of Storage Technology, which raised close to \$100 million for a research and development partnership that was to produce a mainframe computer to compete with that of IBM.

Nelson Schneider of E.F. Hutton says that another factor behind the poor reception for research and development partnerships in biotechnology is that there is now much more competition for the investors' dollars. "There are literally dozens out there in the market in the \$2-10 million range in biotechnology", he says.

Stephen Budiansky

Research councils defend manning

SIR Hermann Bondi, chairman of the Natural Environment Research Council (NERC), appeared before the Public Accounts Committee of the House of Commons on 14 May to give a doughty defence of his council's manpower planning. The National Audit Office recently criticized research councils and the Department of Education and Science for apparent laxity in their planning controls (see *Nature* 309, 104; 1984), and suggested that there had been an unexplained upward drift of salary grades at councils including NERC. But, according to Sir Hermann, the increase in the number of senior posts was a planned development and a completely appropriate outcome of the changing role of NERC.

Sir Hermann said that over the past 10-15 years the need for lower-grade NERC staff on foreign aid programmes had decreased, as local inhabitants had been trained to take over their jobs. The simple result was that the staff NERC retained were now in higher grades. He also asserted that NERC institutes such as the Institute for Marine Environmental Research were deliberately established with young scientists who would climb up the ladder of promotion.

Sir Hermann also pointed out that scientific equipment had become more complicated, so that many measurements that

were formerly made manually are now made by computer. This also led to a smaller requirement for lower-grade support staff.

He went on in stirring style to turn the tables on MPs who had been led to doubt whether the research councils were discharging their manpower planning duties effectively. The main charge of the National Audit Office was that the research councils had not been keeping their staff grades in line with those in the civil service. The Public Accounts Committee was particularly worried about "fluid grading" of science research posts. Sir Hermann's ingenious reply was that manpower planning in NERC is better than that in the civil service. The in-house system of visiting groups, promotion boards and annual reviews was all that could be desired.

The committee's next witness, Dr Ralph Riley, secretary of the Agricultural and Food Research Council, said that the controls on manpower in his council were similar to those at NERC, so much the same arguments applied.

The committee appeared well pleased with these answers. But research councils have not escaped completely unscathed. Mr David Hancock, permanent secretary at the Department of Education and Science, used the occasion to announce a "thorough review" of fluid grading.

Tim Beardsley

Australasian science

Going all the way with B.J.

Canberra

THE 54th Congress of ANZAAS, the Australian and New Zealand Association for the Advancement of Science, was held amid the fading autumn colours and falling leaves of the spaced-out campus of the Australian National University. For five days from 14 May, the congress generated wide coverage of popular items in the press and on radio. Yet the congress lacked the focus promised by the stated theme of "Horizons of Science". The congress was woolly at the edges and soft at the centre.

For decades, the peripatetic congress has struggled to resolve the inherent tension of providing both parallel specialist meetings and cross-disciplinary exchange. Other conflicts surfaced again, notably the confusion about objectives. Is ANZAAS primarily seeking to communicate the latest results, ideas and challenges in science to the wider public and, incidentally, to persuade politicians of the importance and relevance of science? Or is the event principally for academic discourse? At Canberra, neither promise was fulfilled.

Among the 1,600 delegates — a big drop from earlier meetings but still the largest meeting on the Australian academic calendar — were relatively few who had come to listen and to discuss. With more than 1,000 papers being delivered, the speakers outnumbered others. People from outside the academic or research spheres were notably absent. To reach the public at large, or even groups such as schoolteachers and students, ANZAAS relies entirely on journalists.

The trend away from science and technology towards the social sciences and humanities continued. Of the 33 specialized sections, only 15 were devoted to the sciences and technologies, and some were pitifully weak.

The "Horizons of Science" theme, only dimly reflected in the congress programme, was almost totally ignored by individual speakers. Thus a showcase for the current state of Australian science was left empty. But some of the congress-wide symposia produced fruitful discourse, tackling issues of current concern in Australia which as exotic diseases, science and political decisions, technology strategy and nuclear arms. The last, with Petra Kelly and retired General Gert Bastian, parliamentary members of the West German Green Party as visiting attractions, was the best attended, reflecting the growth of the peace movement in Australia.

This symposium even produced a collective statement by a working group on the effects of the British nuclear tests carried out in the Australian desert between 1952 and 1963. Allegations of a cover-up by the British of aspects of these tests have become the centrepiece of a

major diplomatic row between the Australian and British Governments and the rallying point for anti-nuclear protests in Australia. The ANZAAS group called on the Australian Government to conduct a comprehensive interdisciplinary study of the effects of the British tests, saying that a committee formed by the Department of Resources and Energy is inadequate.

ANZAAS was not entirely at odds with the federal government, however. Professor Sir Gustav ("Gus") Nossal, this year's president, gave one of the most glowing tributes to a politician by a scientist in recent times. An immunologist and director of the Walter and Eliza Hall Institute for Medical Research in Melbourne, he solidly backed the discussion draft released in April by Mr Barry Jones, Minister for Science and Technology (see *Nature* 3 May, p.6).

The draft, he said, was "the most significant and courageous document to come out of Canberra on any topic in the past decade". The ANZAAS president went on to urge that the minimum realistic incentive for industry to increase its commitment to research and development would be a tax credit of 150 per cent.

Sir Gustav saw "the most significant, marvellous and stunning element" in the technology strategy draft as being the proposals for achieving 50 per cent completion of secondary school (from 36 per cent now) and 20 per cent of tertiary education (a doubling of the present figure) by 1995. While critical of the emphasis on the quantity of education and of the view that the draft was "singularly silent on quality", Nossal has put his reputation on the line as an influential backer of Jones.

Not everybody in Canberra approved. Professor Gordon Reid, until recently at the University of Western Australia and soon to be governor of the state, said Sir Gustav's statement was not based on scientific inquiry and accused him of "exercising hyperbole in his role as president".

Professor Reid said Sir Gustav's statement was an example of the ambivalence of Australian science. In one breath, he said, scientists plead for freedom from government direction, and in the next for more financial support.

Nossal is unrepentant. "A tremendous challenge is faced by the Australian scientific community to explain ourselves better. . . and to promote a technological culture. We scientists should inform the public about what we are doing, about what practical results can realistically be expected, and about what longer-term dreams can legitimately be espoused."

Later, he complained of "the besetting sins of Australian scientific academics of treating PhD students as clones of themselves and of shunning applied orientations for their research". He asked university

administrators to treat their relations with the media and the public "seriously and as a sacred duty".

By defining its role in terms of facilitating communications among scientists and between scientists and the public, ANZAAS might be expected to take a lead in solving the problems posed by its current president. In reality, ANZAAS has little to offer between congresses as its central organization is weak and strapped for cash (it receives no government support and has fewer than 3,000 subscribing members).

However, bold plans for change were announced by the organizers of the next congress, to be held in Melbourne in August 1985. They will virtually abandon the specialized sections and, through a broad grouping of interests under seven headings, will redirect their offerings to young people and the general public (both of which groups were noticeably absent from the Canberra congress) while attracting scientists to interdisciplinary sessions which will explore the current "state of the art" and identify promising new directions.

Peter Pockley

Nature banned (1937)

AS part of the continuing availability of German documents of the 1930s, it has come to light that the National Socialist government issued a decree, circulated to all university and research libraries, requiring that copies of *Nature* should not be made generally available. Moreover, the decree required that its own existence should be secret.

The text of the decree, dated 12 November 1937, is as follows:

Articles are often published in the London weekly scientific journal *Nature* containing outrageous and vile attacks on German science and the national socialist state. The journal must therefore be excluded from general use in scientific libraries. I request the university, academies, institutes and seminar libraries to carry out the appropriate steps. Regarding the sequestration and restricted use of the journal I refer to my decrees of 17 September 1934, 3 April 1935 and 16 December 1936, UI 22733, W Ie 828 and W Ia 2305.

The decree is signed by the Imperial and Prussian Minister for Science, Education and Development and no doubt refers to the continuous stream of hostile comment appearing in *Nature* in the mid-1930s, much of it centring around the work of the Academic Assistance Council formed in 1933 to help German academics to find posts overseas. Thus in July 1937, *Nature* (140, 169; 1937) referred to the dismissal of German Jewish academics as the "German disaster" and remarked that it was unlikely that "the leaders of intolerance and irrationalism will be deterred by the knowledge that their victims will be sheltered in the asylum of liberal countries". □

Indian science circus

SIR — While your comprehensive reports on science in India in the issue of 12 April are true and appropriate, I should like to stress some of the basic problems behind the prevailing confusion afflicting the Indian science scene.

Modern scientific education and research struck roots in India during the early twentieth century and rapidly grew after independence. The first scientists who were patronized by the government had their own individualistic ideas on developing science in India, for which most of the equipment had to be imported. Soon after independence, India suffered a shortage of foreign exchange, which hindered the import of adequate support materials, but the number of scientific institutions nevertheless continued to increase. This odd situation led to a lowering of the standards of scientific activity. However, many students could imbibe bookish knowledge and some of them could easily migrate to other countries where opportunities for work were abundant. Thus, Indian scientists are thriving both at home and abroad, but occasional soul-searching goes on and comparisons between home-based and expatriate Indians are sometimes not well chosen.

It requires a great deal of patience and faith to work in Indian conditions, in which even essential items are perpetually scarce. Time and efficiency are of relatively little importance in our country and hero worship, blind or forced, is part of life. Unfortunately, modern technologies need less hero worship and more efficient practice. However, our policy-makers (heroes) are not deterred from buying or borrowing any latest technology, and camp-followers are easily found among our vast multitudes. Thus, India constitutes a country of tremendous contrasts wherein basic facilities such as power supply, telephone and other communications services are woefully inadequate on the one hand, while on the other, huge installations for research in space and biotechnology are being planned without the least hesitation. Just as the highly rich and the abjectly poor exist side by side, so also do high and low technologies. After all, ancient emperors built great monuments like the Taj Mahal, probably employing a starving labour force, and as if to keep up that tradition, our science administrators in the past few decades have also been able to build a few glamorous edifices in the name of excellence.

Our tolerance is a boon, giving us the willpower to live amidst glaring disparities, but it is a bane to the implementation of contemporary developments. Finally, it is another wonder of the world to watch the mystic religious practices that cost next to

nothing and modern scientific pursuits at exorbitant costs blissfully coexisting in India!

C. RADHAKRISHNAMURTY
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No thanks to. . .

SIR — So you think that the Royal Society "has broken new and dangerous ground" in the acknowledgements by authors of papers published in the society's journals (*Nature* 26 April, p.762). What is so new? And why so dangerous?

The practice of acknowledging help from others goes back a long way, even in this society's history, so it is certainly not new. If authors are now a little more generous and a little less formal than in the past, why is that so dangerous? The law of libel should remind editors not to allow to be printed the sort of remarks that you fear could be dangerous.

A paper in *Phil. Trans.* (1883) concludes "My best thanks are due to Professor Lankester for some excellent specimens of *Paragorgia*, *Villogorgia* and *Briareus*, and I am also deeply indebted to Professor Mosely who freely placed his numerous preparations at my disposal and whose constant aid and advice have been of invaluable assistance to me." The acknowledgements from *Proc. R. Soc.* (1984) that you quote are in a long and honourable tradition.

R.W.J. KEAY

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The Royal Society,
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● Lankester's organisms are Alcanarians (sea anemones). The more recent acknowledgement that seemed to break new ground read "This research was not supported by any military organization". — Editor, *Nature* □

Publishing chronology

SIR — We read with interest the piece by John Maddox under the title "Extinctions by catastrophe?" (*Nature* 19 April, p.685). The complex of problems produced by the distribution of preprints and by pre-publication press coverage is certainly something we should all ponder. It is clear that the system is sometimes beneficial and sometimes not and that there are no simple answers.

In condensing a complex chronology, however, one non-trivial item was left out. In August of 1983, we presented a summary of our research on periodic extinction at a widely attended meeting on the "Dynamics of Extinction", held in Flagstaff, Arizona. The oral presentation was accompanied by a printed abstract as part of the official programme for the Flagstaff meeting. The meeting was

attended by several science writers and subsequently *Science* (221, 935; 1983), *Science News* (124, 212; 1983) and several other publications carried accounts of the meetings that included substantial treatment of our extinction analysis. It was mostly from these accounts that physicists, astrophysicists and geologists heard of our work, and this led to many requests for more information and for preprints. We were unable to send preprints until early October when the manuscript was complete and ready for submission to *Proceedings of the National Academy of Sciences*.

In view of the foregoing, we do not think that the situation leading to the publication of the five papers on extinction in the 19 April issue of *Nature* fits the classic description of scientific communication-by-preprint. Notice of the research results (including the Flagstaff abstract) was available to all readers of *Science* and *Science News* and to all participants at the Flagstaff meeting. Preprints were sent out only later and then were largely limited to those requesting them. Of course, no preprints were released to the press.

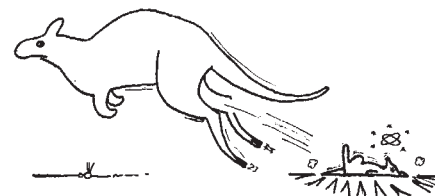
We hope that the publication of this letter will clear up any confusion over this curious incident. DAVID M. RAUP

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Directional pouches

SIR — I was fascinated by the photographs of a gaping thylacine ("Tasmanian tiger") in the series from Jon Darius's *Beyond Vision* (*Nature* 307, 411; 1984). However, among the curious features of the thylacine, the rear-opening pouch is by no means unique. According to Walker's *Mammals of the World* (1964), the posteriorly opening pouch is typical of the bandicoot family Peramelidae and, when a



pouch is present, of the family Dasyuridae. The pouch also opens backwards in the koala *Phascolarctos*, the wombat *Phascolomis* and the marsupial mole *Notoryctes*.

I presume that in most of the quadrupedal marsupials, as in the thylacine, young in a front-opening pouch might be liable to damage by undergrowth, or in the mole, from the pouch filling with soil. Only in the bipedal macropods would a backward — and hence downward — opening pouch have obvious disadvantages.

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Anatomy of a pressure group

from Stephen Budiansky

Mr Jeremy Rifkin's assault on genetic manipulation seems to be succeeding. The danger is that he will not be taken seriously.

IF it were not for the prominent names that keep popping up on his manifestos, people would probably ignore Jeremy Rifkin, the self-styled social crusader who hopes to save mankind from genetic engineering. Rifkin's remarkable knack at canvassing for credibility is once again in evidence, in the case now before Judge John Sirica in Washington, DC in which Rifkin is seeking to halt the first field trials of genetically-engineered bacteria. And it is hard to dismiss one who can wave affidavits from respected scientists who appear to support his claims that serious unfathomed risks are lurking behind these unprecedented experiments.

Combining frequent references to his gallery of the famous with a stream of eminently quotable apocalyptic, Rifkin has succeeded in conjuring an image of genuine controversy. The fact that scientific opinion runs virtually unanimously against him and that his "support" from the famous proves time and again to be far less than meets the eye tells much about public relations on complex scientific issues and about the political naivete of too many scientists.

Crop protection

The case now before Judge Sirica deals with an experiment approved by the Recombinant DNA Advisory Committee (RAC) in April 1983 in a decision remarkable at the time only for its seeming total lack of controversy. Two University of California researchers, Steven Lindow and Nikolas Panopoulos, were hoping to demonstrate the ability of genetically-altered bacteria to protect field crops against frost damage. Under natural conditions, certain strains of wild bacteria that colonize the leaves excrete a protein that serves as a nucleation site for ice formation at a temperature slightly below freezing. If it were not for this protein, temperatures could drop as low as eight degrees Fahrenheit below freezing without frost actually forming. Lindow and Panopoulos believe that by displacing these wild bacteria with laboratory bacteria that have been genetically altered so that they cannot produce the protein, the growing season could be extended by several weeks in the spring and fall. A single gene is responsible for the protein, making this an obvious candidate for genetic engineering.

Although the proposal by Lindow and Panopoulos was RAC's first request for permission to release a genetically engin-

eerred microbe outside the laboratory, it seemed to raise no real safety questions. The researchers had already performed identical experiments with chemically-mutated bacteria — experiments which, if anything, were more dangerous yet which did not require RAC's approval as they did not involve recombinant DNA techniques. The field tests with the engineered bacteria were to be done at a single site, geographically isolated from California's citrus and fruit crops, and the bacteria were to be pre-screened in a greenhouse against any tendency to damage plants. After hearing the report of an outside consultant, Ann Vidaver, a plant pathologist at the University of Nebraska, and noting that the researchers had altered their experimental proposal in response to minor criticisms made earlier by RAC, RAC unanimously approved the experiments.

The experiments were to begin in November 1983. On 14 September, Rifkin filed suit in US District Court, arguing that RAC's approval was invalid because no environmental impact statement had been filed, a requirement for all federal actions having a "significant" effect on the environment. Rifkin also threatened to file for a temporary injunction against the University of California to halt the field trials pending a ruling on his lawsuit.

Lindow and Panopoulos postponed their experiments until this spring, by which time, they hoped, Sirica would have disposed of the matter. Last week, following Lindow's declared intention to proceed with the experiment later this month, Sirica granted Rifkin a preliminary injunction, halting the experiments until he could hear the full case (see p. 296).

Suing the government, of course, has become standard practice in the environmental movement. Key environmental legislation of the 1970s granted citizens an explicit right to actions; other legislation allows those who sue the government in the public interest to collect attorneys' fees if they win. This mechanism has proved a valuable remedy for foot-dragging by the government and has served to ensure that government agencies follow proper procedures.

But suing the government is also a great attention-getter. In the present case, Rifkin has not taken advantage of repeated opportunities for public participation in RAC's deliberations (saying he was out of town on those days). The nuisance-to-substance ratio of the lawsuit is high — at best,

a final decision in Rifkin's favour will require only that RAC go through the formality of producing an Environmental Impact Statement for deliberate release experiments; after that, it can approve whatever experiments it wants without further judicial review. But a barrage of hyperbolic press releases accompany the suit, calling RAC "grossly irresponsible" and accusing it of "reckless disregard for the protection of the environment". In an interview, Rifkin said that the release of genetically-engineered organisms to the environment may "deplete the life-support system of the planet".

Scientists' support

Such statements, of course, make good copy. They also make scientists groan. But Rifkin's ace is the collection of experts he has corralled. "You can dismiss me", he says when challenged about the soundness of his more extreme comments, "but it's very hard to dismiss Peter Raven".

Peter Raven, director of the Missouri Botanical Garden, professor at Washington University and member of the National Academy of Sciences, is probably the most prominent name on Rifkin's list of scientific supporters. His affidavit, like the others, speaks mainly in generalities about the dangers of introducing new species into established ecosystems, and seems to betray an ignorance of the details of the Lindow experiments.

Remarkably, Raven freely admits that he had seen neither the detailed experimental proposals nor RAC's minutes before filing his affidavit. And he said, "I'm not criticizing RAC at all. I haven't even got a position more than to say we need visible discussion." Asked why he did not use RAC's public meeting or the nearly year-long opportunity to file comments with RAC, he said he had "no way of knowing" how he could participate in RAC's deliberations. "Nor was my opinion sought." Raven said the burden must be on RAC to reassure the ecological community that adequate scrutiny was given to the proposal.

Eugene Odum, an ecologist at the University of Georgia, also filed an affidavit. He said he was voicing his concern over the lack of proper procedures for evaluating deliberate-release experiments, not the Lindow experiment itself. "I would doubt that the experiment itself is dangerous", he said, "but it could establish a precedent that's very dangerous."

Yet the procedures are already in place for evaluating deliberate-release experiments. Maxine Singer, a researcher at the National Institutes of Health (NIH) who has been a strong voice for caution since 1973, says that tactics such as Rifkin's lawsuit are unlikely to bring about a more thorough scrutiny of genetic engineering research. "The evidence is there that RAC asks difficult questions and is not about to give permission at the snap of their fingers", she says. And everyone, from Jeremy Rifkin to the biotechnology industry, agrees that, although compliance with RAC's decisions is technically voluntary for private industry, no company would dare proceed without RAC approval, since a company's failure to abide by the guidelines and RAC's decisions would be very damaging evidence of negligence if the company were sued for damages arising from an experiment. And Singer doubts whether the Environmental Protection Agency could command the political clout and scientific respect of RAC.

David Baltimore of Massachusetts Institute of Technology speaks for many when he says that "there are aspects of the direct-release experiments that raise problems that RAC previously didn't have to deal with. But I don't think it's helpful to make generalizations. You have to think carefully about each case. You can't do it on the basis of what you hear from Jeremy Rifkin or what you read in the *New York Times*." And he warns of the dangers of a backlash against Rifkin's tactics: "The continual opposition to recombinant DNA activities has made the scientist community leery of raising even those concerns they feel are legitimate, because they can so easily be taken advantage of. And that's a terrible climate. But it's the inevitable consequence of politicization."

Endangered humanity

This lawsuit is not the first occasion that Rifkin has pulled credibility out of a hat. Last June, after canvassing religious leaders across the country, Rifkin produced a petition calling for an "immediate" ban on engineering of the human germ line. Joining the call were 21 Catholic bishops, leaders of most major protestant sects in the United States, several Jewish leaders, the Moral Majority's Jerry Falwell and television preacher Pat Robertson. A "theological letter" accompanying the position provided the apocalyptic rhetoric — calling engineering of genetic traits "a fundamental threat to the preservation of the human species as we know it". The letter claimed that "it will soon be possible to carry out such procedures on humans, and argued that even the elimination of defective genes — such as those responsible for sickle-cell anaemia — would dangerously narrow the human gene pool and "might ultimately lead to the extinction of the human race".

Rifkin concluded by equating genetic

engineering with eugenics ("the inseparable ethical wing of the Age of Biotechnology") and by calling upon society to oppose human genetic engineering "with the same courage and conviction as we now oppose the threat of nuclear extinction".

Once again, there was less than met the eye. The religious leaders who appeared at Rifkin's New York City press conference were careful to disavow the "theological" letter; they had signed only the much more moderately-worded petition. Subsequent interviews revealed that many of the signatories had minor or even major reservations about the petition as well. Some said they did not oppose treatment of disease, and had signed only to express their agreement with Rifkin's warnings about the misuse of genetic engineering for eugenics. Many reaffirmed their support for somatic-cell gene therapy for the relief of human suffering and the treatment of disease.

Once again, Rifkin may also have alienated those who might otherwise have been his natural allies. Alexander Capron, who directed the President's Commission for the study of Ethical Problems in Biomedical and Behavioral Research — which, until its demise last year, produced a series of carefully argued and widely praised studies of ethical issues in medicine, including genetic engineering — called the petition a "knuckleheaded, broadside" attack that swept aside a host of subtle ethical dilemmas in favour of polemics.

John Fletcher, an Episcopal minister who, as assistant to the director of NIH for bioethics, has thought long and hard about these issues, was also irked by the petition. He wrote to Bishop John Allin, the presiding bishop of the Episcopal church in the United States and a signatory of the petition, criticizing its "apocalyptic" tone and its failure to take into account the "thinking that had already been done". Fletcher said he wishes the signatories had "asked for help to become informed on the body of debate that already existed. It is interesting that the President's Commission report [*Splicing Life*, produced at the request of the major religious organizations in the United States] had existed for a year, and many [of the petition's signers] had not seen it."

Fletcher, significantly, is one of those who have urged caution in proceeding with germ-line alterations, arguing that society at present lacks the necessary ethical and moral system to deal with the consequences of man's engineering his own heredity. "But I am in favour of keeping an open mind", he said.

Others point out that "this is not something that is happening suddenly", as Dr French Anderson, chief of the Laboratory of Molecular Hematology at NIH and a leading researcher on genetic blood disorders, put it. Anderson says although the genes necessary for treating some 20 inherited disorders have been isolated, "we

can't get them into the target cells, we can't regulate them properly and we have no way of assessing their safety" in humans. When it becomes a scientific possibility, the real ethical questions may have little to do with somatic versus germ-line alterations but — as pointed out by the President's Commission report — whether the alteration is reversible (should some unforeseen danger later arise), whether it is aimed at correcting recognized genetic disorder or at "perfecting" humans.

Regulation

In the United States, the regulatory mechanism is also already in place to assure that germ-line manipulation is not rushed into without considerable deliberation. Experiments on human subjects will have to be cleared by a local Institutional Review Board charged with protecting human subjects; the board must include at least one non-scientist and at least one scientist not affiliated with the institution. Human gene therapy trials would also have to be cleared by a similar local panel, the Institutional Biosafety Committee, charged with overseeing recombinant DNA research. The full RAC would then have to give its approval and, before it could be applied routinely in clinical practice, the Food and Drug Administration would have to approve its safety and effectiveness.

The case of Martin Cline, a researcher at the University of California, Los Angeles, who performed unauthorized (and unsuccessful) gene therapy experiments on two patients suffering from beta-thalassaemia, a fatal genetic blood disorder, was ample proof that these regulations are not merely cosmetic. When Cline's experiments became known he was stripped of federal research grants and found his research career effectively ruined.

Just as with his lawsuit, the net result of Rifkin's petition may have been polarization — and a backlash against sensible proposals. Congress, for example, is seriously contemplating the establishment of an advisory body, along the lines of the President's Commission, to monitor developments in human genetic engineering. The concept, championed by Representative Albert Gore, is strongly backed by the President's Commission itself, Capron, Fletcher and many others concerned about the ethical and religious implications of genetic engineering.

RAC, the other logical body to exercise oversight on human genetic engineering, has also recently proposed expanding its membership beyond researchers and physicians to include ethicists.

But, as Capron says, there is a danger that those in research and industry who might otherwise support these proposals may come to see them as "part of the Rifkin attack". The danger is that the very concerns that Rifkin claims to represent may be swept aside, resulting in an all-out political battle between two polarized camps. □

Chaos — a model for the outbreak of war

from Alvin M. Saperstein

The chaos as formulated for physical dynamic systems is applied to a very simple nonlinear model of the arms race. The transition, from stability to instability, from arms race to war, could be analogous to the transition from a laminar to a turbulent (or chaotic) flow.

SIMPLE mathematical models of the arms race have aroused interest among academic physicists¹. Two common models are the dynamic model of Richardson^{2,3} and the static inequality model of Kaye^{4,5}. Both are deterministic: given the model and its parameters, a reasonable estimate of present conditions allows prediction of the future outcome of the arms race. Here I offer the beginnings of another model based on recent advances in the understanding of chaotic motions of dynamical systems^{6,7}. I suggest that war be viewed as a breakdown in predictability: a situation in which small perturbations of initial conditions, such as malfunctions of early-warning radar systems or irrational acts of individuals disobeying orders, lead to large unforeseen changes in the solutions to the dynamical equations of the model. There is no way then to predict the effect of the actions of any participant — analyst, planner, statesman or general — with any certainty. Certainly war, between roughly equivalent parties, has often been characterized by uncertainty in outcome⁸; nations which start wars in great confidence very often end defeated⁹. Typically, in the period immediately before the outbreak of a war the nations attempt desperately to claw back as they slide over the precipice, although they by this time already have lost control of their own destinies¹⁰; control and predictability are lost.

There is a current view that entrance into war, and the war itself are deterministic — escalation, up or down, nuclear or otherwise, can be controlled^{11,12}. But most historical evidence and contemporary analysis indicates otherwise: predictability and control are lost at the threshold between peace and war¹³, just as they are lost when a fluid makes the transition from laminar to turbulent flow.

Here I sketch the meaning and development of chaos in nonlinear dynamical systems and apply these concepts to a simple nonlinear arms race between two nations. Both the model and the illustrative parameters are admittedly over simple and do not as yet apply to the real world, but I hope that the concept of 'chaos', as used herein has a bearing on the present world situation. I believe that nonlinear dynamical models, with their capability for both predictable and non-predictable orbit through the phase space of international competition, are necessary for realistic understanding of the present

arms race.

Deterministic models patterned after those used successfully in physics have previously been applied to the description of competition in economics and biology¹⁴. Because these models may also have a significant nonlinear component, it follows that 'chaos' may also result from these competitions; some examples have been reviewed by May⁷.

Richardson's model for the arms race between two competing states consists of a pair of coupled first-order linear differential equations. The independent variable is time and the dependent variables are measures of the devotion of the two societies to hostilities — expenditure on arms, percentage of population in the military, and so on. The coupling terms, the off-diagonal elements of the matrix of the system of differential equations, represent the reaction of each nation to the challenge of the other: thus the rate of change of the armaments of nation A is assumed proportional to the total armaments of nation B and vice versa. The diagonal terms of the system represent the intrinsic reaction of each nation to its own armaments; for example, a large military establishment might successfully press for further increases in military expenditure, or the rest of society resist increases in the share of resources going to the military.

The solutions of such a model system are simple linear combinations of exponentials in time. The exponents are determined by the model parameters whereas the coefficients of the linear combinations are uniquely determined by the initial conditions. Small changes in the initial conditions imply small changes in the coefficients, so that two solutions which start very close together will always remain comparatively close together. This is the sense in which the behaviour over time of a dynamical system is predictable. Knowledge of initial conditions is always uncertain, which means that a set of orbits, or a 'flow', is allowed by any practical model. Since all orbits in the Richardson model flow remain close to each other, practical predictions remain possible.

If the model parameters are such that the exponents are negative, the solutions damp down to an equilibrium value no matter how starting conditions are chosen. Such a case is 'stable'. If the model parameters imply positive exponents, the flow departs exponentially from any starting conditions

and the armaments of both parties increase exponentially. This situation is 'unstable' and may imply war or the certainty of eventual war.

A peacemaker in Richardson's model must alter the parameters of the competition so that only negative exponents are possible. If he accomplishes that, he can successfully predict peace (or at least inventories of the tools of war which do not grow) no matter what the starting configurations are. Conversely, if the parameters of the model lead to instability, the generals can know what to expect, given reasonable intelligence of the starting configuration.

The Richardson model is dynamic, relating changes in the configuration to a present configuration, and stability or instability to the kinds of change possible. By comparison, the Kaye model of bilateral strategic deterrence^{4,5} is static, dealing with the two parties' perceptions of their situation. The variables are the numbers of nuclear warheads each is capable of firing at the other, the model parameters are the dependability, accuracy and destructive capabilities of these warheads. There is no time parameter or variable.

Given one set of inequalities between the variables, one side can punish the other without undue loss. That side is, therefore, secure and can afford to relax; the other side perceives no such security. Given another range of inequalities between the same variables, both sides are assured that they can inflict unacceptable damage upon their opponents even after suffering the worst first-strike devastation their opponent is capable of delivering; both sides perceive 'mutually assured destruction' so have no incentive either to launch a first strike or radically to change their present state of armament. This represents 'stability' or 'stable deterrence' in the Kaye model. Finally, the range of inequalities remaining includes the situation where each side can destroy the other so that the side that strikes first can prevent its victim from retaliating: each side has an incentive to strike first or radically to increase its armaments. The situation is unstable or is said to represent 'unstable deterrence'.

The Kaye model is described by a Cartesian plot in which the two orthogonal axes represent the number of warheads or launch vehicles available to each of the two sides. The inequalities are represented by different regions of the plot. For example, in Fig. 1, adapted from ref. 5, the number of

launch vehicles possessed by side A, N_A , is plotted against N_B , the corresponding number for the opposing side B. In the region designated DA, A is dominant; it can punish B with a successful disarming first strike without fear of severe loss to itself. Similarly B is dominant in region DB. Region MAD is the stable region of mutually assured destruction whereas FS is the unstable deterrence region in which each side has the incentive to strike first. The remaining region, IN, represents that in which neither side has sufficient warheads to do conclusive damage to the other. Thus there are stable and nonstable areas which, topologically are simply connected and have simple boundary curves. Except for the boundary curves themselves, nearby points in the plot belong to the same region. Thus small uncertainties about the actual numbers need not lead to changes in the perception of stability or instability. In this sense, the static model also permits practical predictions.

In physics, we are accustomed to assuming predictability based on Newton's equations of motion. Given the forces, specific initial conditions lead to specific well-defined orbits in an appropriate phase or coordinate space. Similar concepts lead to the nonlinear equations of fluid dynamics. Since actual fluid motions exhibit both laminar and turbulent flows, we must accept that the solutions of the equations of fluid dynamics may represent either laminar or turbulent character and that the nature of the flow changes from laminar to turbulent as some parameter or combination of parameters increases through some critical value¹⁵.

Laminar flow implies predictable behaviour: streamlines which start off near to each other remain near to each other. Knowledge of the motion at one point in the flow at one point in time implies knowledge of the motion at neighbouring points in space and time. Turbulence, by contrast, is chaotic: knowledge of the motion at one location at one time says nothing about the motion at nearby points at the same time or at the same point at later times: prediction is fundamentally impossible. Hence the same mathematical model (the same set of equations) allows predictable behaviour in some regions of a model parameter, and unpredictable behaviour in others. This breakdown of predictability in certain systems seems a good characterization of war in those systems.

Civil life presupposes a major component of predictability. The formulation and carrying out of policy requires a reasonable ability to predict the future given some knowledge of the present. The same requirement for predictability holds for the relationships between nations, whether they are engaged in normal international competition or in a hostile arms race. But the history of nations is full of examples of a loss of control, by one or more governments, as they make the transition

from peace to war. Thus any single model of an arms race between nations should include regions of predictable behaviour, regions of chaos and transitions between such regions.

As a simple model of the transition from predictability to unpredictability consider the flow represented by the sequence of mappings⁶:

$$x_{n+1} = 4bx_n(1-x_n) = F_b(x_n) \quad (1)$$

where $0 < x_n < 1$, $n = 0, 1, 2, \dots$ and $0 < b < 1$. A specific orbit is the succession of values $x_0, x_1, x_2, \dots$ starting from a specific value of x_0 . A flow consists of a set of orbits, starting from a set of neighbouring values of x_0 . The flow is predictable if its constituent orbits remain near each other; it is unpredictable (chaotic) if they drift apart exponentially: if, given any x_0 and x_0' , the difference between x_n and x_n' diverges exponentially as $n \rightarrow \infty$. No matter how small the subinterval ε is from which a chaotic flow starts, it soon fills the entire interval $(0, 1)$; no matter how accurately the starting value x_0 is measured if the motion is chaotic knowledge of the position of x_n is soon lost — it is distributed with equal probability throughout the allowed region $(0, 1)$. Prediction is impossible for a chaotic flow: the flow determined by (1) above changes from predictable to unpredictable as b increases through the critical value $b_c = 0.892$.

Let x^* be a fixed point of a flow, F , defined by the equation $x^* = F(x^*)$ (2) Then x^* is a stable fixed point if

$$F'(x^*) = \left| \frac{d}{dx} F(x=x^*) \right| < 1 \quad (3)$$

If x^* is a stable fixed point, it is easy to see⁶ that all orbits, no matter where started, tend to x^* as $n \rightarrow \infty$. Thus given such an x^* , perfect prediction is possible: wait long enough and any flow converges to x^* . It is easy to show that $x_b^* = 0$ is the only stable fixed point of F_b for $b < 1/4$. For $1/4 < b < 3/4$, the only such point is $x_b^* = 1 - (4b)^{-1}$. And for $b > 3/4$, there is no stable fixed point of $F_b(x)$.

Consider now the mapping $F_b^{(2)}(x) = F_b(F_b(x))$ and its fixed points x_b^{**} . If x_b^{**} is not a fixed point of F_b , it follows that

$$F_b(x_b^{**}) = \bar{x} \text{ and } F_b(\bar{x}) = x_b^{**}$$

where $\bar{x}$ does not equal x_b^{**} , (note that x is also a fixed point of $F_b^{(2)}$). Thus $x_{n+2} = x_n$, $x_{n+1} \neq x_n$, if $x_n = x_b^{**}$. It follows that the flow F_b oscillates back and forth periodically between pairs of stable fixed points of $F_b^{(2)}$, if these exist while those of F_b do not. Such a situation occurs for $3/4 = b_1 < b < b_2 = 0.862 \dots$. Again, prediction is possible, though more complex.

For $b > b_2$, the fixed points of $F_b^{(2)}$ are no longer stable, so that it is no longer possible to say that the flow converges to a periodic oscillation between two points. But there is now an interval $b_2 < b < b_3$ for which the fixed points of $F_b^{(4)}(x) = F_b^{(2)}(F_b^{(2)}(x))$ are stable. The flow now converges to a periodic oscillation between four points. Prediction is still possible though even

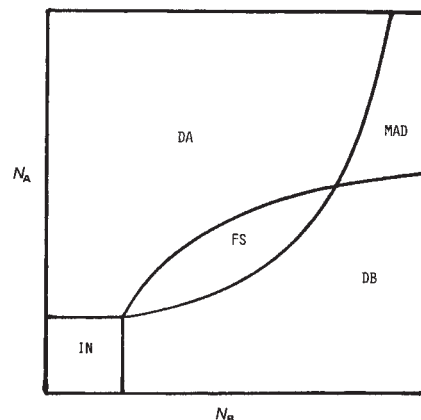


Fig. 1 A strategic balance diagram (Kaye plot) showing regions of stability and instability.

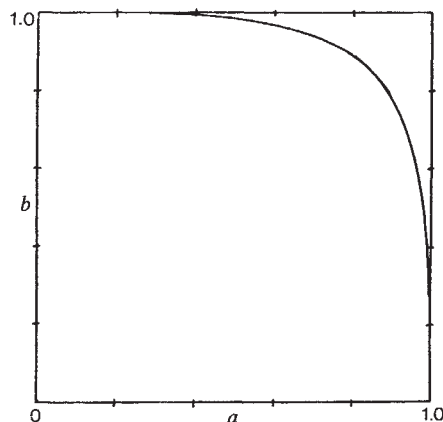


Fig. 2 A curve representing $f_4(a, b) = 0$ (see text), taken to be a reasonable approximation to the critical curve $f_c(a, b) = 0$ separating the region of predictability from that of chaos. Data courtesy of Larry Dishman, Wayne State University.

more complicated. As b increases further, the fixed points of $F_b^{(4)}$ become unstable, the four-point periodic motion disappears and the flow now converges to an eight-point cycle, which then becomes a 16-point cycle and so on.

There is thus a sequence of periodic oscillations of ever-increasing complexity corresponding to a sequence of transition values b_i with an accumulation point at $b_c = 0.892 \dots$. Beyond this value lies chaos: orbits do not have definite end points, initially neighbouring orbits diverge exponentially, any flow soon fills the entire interval and prediction is impossible. In this simple model, b_c corresponds to the critical Reynold's number which governs the transition to fully turbulent fluid flow.

A bilateral arms race between two competing countries X and Y is assumed to occur in stages designated by an integer indicator n ; successive values of n may indicate successive years, successive budget cycles and the like. (Thus n is a discrete version of the independent variable, time, in the Richardson model.) The dependent variables x_n, y_n represent the fraction of the two nations' resources devoted to armaments: $0 < x_n, y_n < 1$. A nation's armaments at the next stage depend upon its perception of its opponent's armaments at the present stage; I will assume x_{n+1} to be proportional to y_n and conversely.

Table 1 Ratio of national military expenditure to GNP

	France	Germany	Italy	UK	USSR
1934	0.0276	0.0104	0.0443	0.0202	0.0501
1935	0.0293	0.0125	0.0461	0.0240	0.0552
1936	0.0194		0.0298	0.0296	0.0781
1937	0.0248		0.0359	0.0454	0.0947

But the perceived threat also depends upon how much of the opponent's resources remain potentially convertible to armaments at a future stage. If all of the opponent's substance is devoted to arms, there is no need to be concerned about a future increase in threat (assuming fixed total resources). Hence I also assume x_{n+1} to be proportional to $1 - y_n$ and conversely. My simple arms race model thus consists of the pair of sequences of mappings:

$$x_{n+1} = 4a y_n (1 - y_n) = F_a(y_n) \quad (4)$$

$$y_{n+1} = 4b x_n (1 - x_n) = F_b(x_n) \quad (5)$$

in which $0 < a, b < 1$. If the values of a and b are such that the flows are predictable the race will be said to be stable. Each party will know what the future portends and can plan accordingly. If the parameters product a chaotic flow, the race is unstable and neither can know what the future will bring.

Analysis of the model with two parameters and two variables represented by equations (4) and (5) is similar to that of equation (1). Fixed points are characteristic of the mapping of a variable onto itself. Thus in this case the appropriate sequences of mappings are not F_a and F_b but, instead,

$$\begin{aligned} F_{ab}(x) &= F_a(F_b(x)) \text{ and} \\ F_{ba}(y) &= F_b(F_a(y)) \end{aligned} \quad (6)$$

Fixed points of these sequences imply

$x_{n+2} = x_n$ and $y_{n+2} = y_n$ and are again stable if the absolute magnitude of the derivative is less than unity. It is easy to show that, for each fixed point x_i^* of F_{ab} , there is a corresponding fixed point y_i^* of F_{ba} ; with $y_i^* = F_b(x_i^*)$ and $x_i^* = F_a(y_i^*)$. Further, if one of the pair of fixed points is stable, then so is the other since the two slopes are equal. A similar relationship connects the fixed points of $F_{ab}^{(2)} = F_{ab}(F_{ab})$ and $F_{ba}^{(2)} = F_{ba}(F_{ba})$, and so on. Hence, there cannot be predictability in one of the variables and unpredictability in the other. Both parties to the arms race will be at war or at peace simultaneously, which corresponds with commonsense.

Again, as the values of a and b are changed, there is a sequence of flows in which first the fixed points of F_{ab} and F_{ba} are stable; then these fixed points become unstable while those of $F_{ab}^{(2)}$ and $F_{ba}^{(2)}$ remain stable; these then become unstable while the fixed points of $F_{ab}^{(4)} = F_{ab}^{(2)}(F_{ab}^{(2)})$ and $F_{ba}^{(4)} = F_{ba}^{(2)}(F_{ba}^{(2)})$ remain stable, and so on. Instead of a sequence of transition points b_i and a critical accumulation point b_c , as above, there is now a sequence of transition curves $f_i(a, b) = 0$ (symmetric in a and b) and a critical accumulation curve $f_c(a, b) = 0$ beyond which the two flows are chaotic.

The actual transition values, a_i , b_i , for the model of equations (4) and (5) have

been estimated graphically. Plots were made, for a sequence of values of a and b , of $F_{ab}(x)$, $F_{ab}^{(2)}(x)$, $F_{ab}^{(4)}(x)$. Intersections with the straight line $F(x) = x$ were determined and the slopes F_{ab} , $F_{ab}^{(2)}$, $F_{ab}^{(4)}$ were measured by eye. A transition was judged to have occurred when the absolute magnitude of one of the slopes exceeded unity. Given the crudeness of these measurements, the critical accumulation curve is assumed to be indistinguishable from the curve $f_4(a, b) = 0$ which determines the transition of $F_{ab}^{(4)}$ from stability to instability and which is sketched in Fig. 2. Chaos, and hence war, occurs for those values of the model parameters a , b lying above and to the right of the curve.

It is tempting to apply this model to previous and present arms races even though the data are difficult to obtain and the model is over-simple. Some data are available for the period immediately preceding the Second World War¹², although the figures are questionable. Nevertheless, it is possible to assign values for the ratio of national military expenditure¹⁶ to gross national product (GNP)¹⁷ (Table 1).

The values in Table 1 are x_0 , y_0 , x_1 , and y_1 (subscripts 0 and 1 designate the earlier and later of the two years used) to be substituted into equations (4) and (5) to obtain parameters a and b for pairs of competing countries in the European arms race during the 1930s (Table 2).

Table 2 The parameters a and b

		a	b
France-Germany	(1934-35)	0.712	0.116
France-Italy	(1936-37)	0.214	0.472
UK-Germany	(1934-35)	0.582	0.158
UK-Italy	(1934-35)	0.142	0.582
USSR-Germany	(1934-35)	1.34	0.0657
USSR-Italy	(1936-37)	0.819	0.125

Thus the German-Soviet arms race was in the chaotic region, and the French-German and Italian-Soviet races were clearly close to the transition region. We all know that the Second World War broke out shortly thereafter, but it is difficult to give much credibility to this *post hoc* calculation because the model is purely bilateral.

Turning to the present-day arms race between the United States and the Soviet Union, I assume for the sake of illustration that 7 per cent of the US GNP is devoted to military and associated expenses, so that $x_0 \approx 0.07$. Assuming that Soviet armament expenditure is comparable with that of the United States and that Soviet GNP is roughly half that of the United States, it follows that $y_0 \approx 0.14$. Given the crudeness of the numbers and the model, it is reasonable to assume $x_1 \approx x_0$, $y_1 \approx y_0$ from which it follows that $a \approx 0.15$, $b \approx 0.54$.

Thus the Soviet-American arms race at present lies within the stability range of Fig. 2. Instability develops when b (or a) approaches unity; this would occur if y_0/x_0 increased from two to between three and four. Given the crudeness of the model and choice of parameters, no comfort can be drawn from this last statement. A more realistic model may require more than one dependent variable for each side. With such additional variables the threshold to chaos may be lower.

The crude model presented here is not a credible representation of world affairs. At the very least, it should be expanded to include the equivalent of Richardson's diagonal terms: the direct affect of x_n upon x_{n+1} , of y_n upon y_{n+1} . Also required is a careful determination of the model parameters, the a and b of the off-diagonal terms as well as corresponding coefficients for the diagonal terms^{18,19}. This latter task would require the accumulation of data for arms expenditures and GNP over a sequence of several years, a task made difficult by the lack of Soviet data comparable in definition and quality with the generally available US data^{20,21}. There is also the problem of the definition of military expenditure in today's high technology world.

Thus any realistic model of a two-party arms race will most probably include nonlinear terms as a result of which there may be a transition from a predictive state of affairs to chaos. The creation and analysis of such chaos-predicting models and the practical applications of the insights they provide can contribute to avoidance of such chaos in real life. □

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Particle people compile data

The latest compilation of particle physics data is a monument not merely to those who discover new particles but also to those who have improved the accuracy of what is known.

THE complaint that there are simply too many material particles for any of them to justify the old adjective "fundamental" may seem, on the face of things, to be fully borne out by the latest compilation of properties by the Particle Data Group, a collaboration of 23 physicists which is an international outgrowth of the old Berkeley Particle Data Group on which responsibility for a critical review of available data used to rest. The new compilation, based on data which had become available by the end of 1983, runs to 304 pages of the *Reviews of Modern Physics* for April this year (56, S1-S304; 1984) and is likely to be many people's bible for years to come. It is also a model of how data should be compiled in a fast-moving field. The moral seems to be that the job is best done by people who are themselves practitioners.

In reality, the compilers have had to break with the traditions of their predecessors and now explain that they can no longer include references to all relevant publications in high-energy physics bearing on the properties of particles. The literature, they say, is growing by about 10 per cent a year, with the result that archival material must rest where it lies, in previous compilations, the most recent of which was two years ago. Laconically, however, the new list of particles includes (under "stable particles", if you please) the measured values of the masses of the W and Z particles first discovered just over a year ago at CERN, the European high-energy physics laboratory.

The classification of these particles (along with the photon) as stable is not as eccentric as it may seem. The criterion is that particles whose decay is accomplished only by the intervention of weak nuclear forces are counted as being stable. By the same test, a whole string of unstable mesons and baryons are thrown together in the same group.

To outsiders, perhaps the most surprising feature of these listings is the comparative speed with which it seems to have been possible to refine the accuracy of the masses of recently-discovered particles. Thus, among the group of three known leptons (electrons, μ -mesons and τ -mesons, each with negative or positive electric charge), the masses of the first two are understandably known to within one part in a million (after eight and four decades respectively), but even that of the τ -meson is known to within 2 parts in 1,000

after less than a single decade. Among particle physicists, it must be tempting to hope for a further order of magnitude in the accuracy with which the mass of each particle is known with the passage of each decade. But this can only be a rule of thumb; the estimated mass of the first strange meson (K), discovered more than thirty years ago, is hardly more precise (at one part in 50,000) than that of the J/ψ , just over a decade old and the first experimental proof that charmed quarks do indeed exist.

In the circumstances, it is something of a surprise that while the mass of the neutron is known (as it should be after all this time) to better than one part in a million, the lifetime of this particle against radioactive decay (into a proton, a negative electron and an antineutrino) is known only to within 2 per cent or thereabouts.

This is not, of course, the scandal that it may seem to be. The experimental difficulties of anything like a direct measurement of a decay whose products are among the most common particles in the real world, and — worse still — themselves stable, are bound to be formidable. But the practical importance of knowing the decay rate of neutrons is far from negligible even in such straightforward circumstances as the production of bursts of cosmic rays from the Sun.

On the lifetime of the proton, the new compilation of particle data is also necessarily unhelpful. The authors can only quote the steadily lengthening list of largely negative searches for evidence that protons do indeed decay, as the grand unified theories uniting electromagnetic, weak and strong forces suggest they should, into, say, electrons and photons. As is well-known, the lower limits on the lifetime determined experimentally are already less than the lifetime calculated by the simplest of the theories, according to this compilation by "at least one order of magnitude". Given that the first experimental data appeared only in 1981, and that nearly a score of substantial papers on the subject have since appeared, it cannot be much more than a year before the decay of the proton is observed, or the theories put in considerable difficulty.

Another of the still-fruitless searches reported in the compilation is that for the sixth quark, labelled *top*. By now, it seems thoroughly established that the particles called mesons consist simply of a quark and an antiquark, not necessarily of the same

breed. In retrospect, it is clear that the most familiar mesons (the muon and pion) and the less massive of the two strange particles discovered in the late 1940s are made only of the three quarks called *up*, *down* and *strange*. *Charm* was found at Stanford University a decade ago, and *bottom* soon afterwards, but *top* remains to be discovered.

This latest compilation of particle data has surprisingly little experimental evidence to report, all of it dating since 1981. And much of this is entirely negative — people have looked for particle resonances over a range of energies and have found nothing to suggest the existence of a *top* quark. But there is a handful of papers that appear to conclude that something happens at an energy of about 34 GeV which could be the signature of the species called toponium, a particle built from the *top* quark and its antiquark. If that is the case, the proton-antiproton collider at CERN should find *top* any day now. Some would say that the missing quark should have been found already.

Even in this dry listing, the search for free quarks is more fun — but equally fruitless. In a listing of more than 250 experimental searches (each of which is solemnly accompanied by a number giving the number of "quark events" recorded), all but a few of the corresponding entries consist simply of the digit "0". The experiments cover both searches for free quarks in accelerator experiments and those, based on the assumption that free quarks could be relatively massive and stable particles, in which people have sought to find quarks by taking vacuum cleaners to the ground beneath electrified security fences. The commentary in the compilation says that "of the several candidate cosmic ray events, one still enjoys the active advocacy of its discoverer".

Other particles have been sought and not found in great profusion. On the evidence of this listing, the magnetic monopole does not exist. From the exotic zoo of particles such as the Higgs boson (which *should* exist if only people knew where to look) and the tachyon (which entails time-reversal), the axion is singled out for special attention in this compilation, no doubt because of the recent popularity of these still unknown particles as plausible constituents of the missing mass in the Universe. But here again, for the time being at least, the experimentalists have drawn a blank.

John Maddox

Auditory physiology

Mobile maps in the brain

from John D. Pettigrew

POINT-to-point matching of an array of sensory cells to an array of central neurones produces retinotopic and tonotopic maps in the brain, which have been the stock in trade of neuroscientists tackling problems such as the control of neuronal specificity. But a neural map of auditory space cannot be generated simply by the equivalent topographical projection from the cochlea at the auditory periphery, because the cochlea represents the frequency of the tonal sound stimulus rather than its location in space. Instead an auditory space map must be computed from subtle cues provided from the effect of the head and pinna on the sound field. It therefore came as a revelation to find a 'computational map' of auditory space in the brain of the barn owl, where individual neurones are specifically tuned to different sound source locations and are arrayed so that position in the brain map is systematically related to the preferred location in space¹. The mechanism by which such a map is generated is still being explored, but meanwhile a study of an auditory space map in the monkey's midbrain has produced another revelation, reported by Jay and Sparks on page 345 of this issue².

Jay and Sparks find that single neurones in the monkey superior colliculus show specificity for the azimuthal location of a sound source and are arranged topographically to form a map of sound space somewhat like that described in the owl, but with one feature that is new and surprising: the coordinate frame of the map shifts along the horizontal plane as the monkey's eyes change position. Thus, for example, a neurone which responds to a sound source located 10° to the right of the eye's fixation point when this is straight ahead will still respond to a source located 10° to the right of the fixation point when the eyes are looking 40° right, even though the head and ears have remained stationary.

The new findings have come from conceptual and technical innovation in the presentation of sound stimuli, which auditory neurophysiologists have at last moved from the ear-canal out into free-field auditory space. This move, like that made by visual physiologists in the late 1950s away from direct retinal stimulation towards the use of natural visual stimuli^{3,4}, avoids assumptions about the way in which complex spatial parameters of the natural stimulus are encoded; and it is proving to be as fruitful in audition as it was in vision.

One of the first free-field auditory stimulators used by auditory neurophysiologists was called Herb's Hoop, after the Caltech craftsman who built it while also working

on equipment for the Viking spacecraft. Appropriately, the hoop was initiated on the same day in 1976 that the Viking lander touched down on Mars, and since that time has been responsible for a succession of important new findings, beginning with the first description of single neurones with sharply restricted receptive fields in auditory space⁵ and their arrangement into a map of auditory space¹.

The finding of the computational map of auditory space in the barn owl provoked a search for a comparable map in the brain of mammals, and 'hoops' sprang up in auditory neurophysiology laboratories from Oxford to Alabama to Monash. This search is still going on in the cat, despite a string of negative findings^{6,7}, and difficulties have been encountered in finding any representation of auditory space in the mammalian inferior colliculus. In contrast, numerous studies have found evidence of spatial representations of a variety of stimulus dimensions, all in register, within the mammalian superior colliculus. Sound space, visual space and even somatosensory space are all mapped with the same rough topography into the superior colliculus of various mammalian species; and maps of auditory space and of the direction of infrared sources are located within the equivalent structures of the owl⁸ and rattlesnake⁹ respectively.

Distortions occur in the course of the generation of maps which vary according to modality. Registration between maps can therefore be a problem and considerable interest has focused on this question. Gross misregistration can be corrected developmentally, as shown in elegant experiments by the Knudsens on young barn owls wearing prisms to displace their visual fields. In this case the map of auditory space shifts to realign itself appropriately with the visual map, whose coordinate frame is unchanged (relative to both the eyes and the head within which the eyes are immobile in owls).

Despite such plasticity, smaller local areas of misregistration still occur and are puzzling if considered in purely sensory terms. If, on the other hand, one considers

the pool of motoneurones generating the orienting response from activation of some part of the map, precision or registration takes on a new meaning. This has been most clearly pointed out by McIlwain^{10,11} who has formulated some rules for representations within the superior colliculus. A very precise orienting response may be driven by a rather diffuse activation of output neurones within the motor map since the precision depends upon co-ordinated activity in a very large pool of different motoneurones. McIlwain has shown how, as the final motor output is approached, the size of the 'point image' activated within the array by a stimulus increases to include a significant part of the whole map.

The point image can be thought of as the spatial distribution of neurones which are activated within a map by a punctate stimulus in the appropriate sensory space. The size of the point image in the map is determined both by the receptive-field size of individual neurones and by the degree to which receptive fields of neighbouring neurones overlap. A remarkable feature of a number of visual maps, first noticed by McIlwain, is that the mapping and receptive fields are related in such a way that the point image at a given map location has the same size as a receptive field at that location when the receptive field is projected onto the map. Accordingly, the very large receptive fields encountered in the deeper layers of the superior colliculus will be associated with large pools of output neurones, to which increasing attention is now being paid by recording from behaving monkeys. Thus, the large and apparently imprecise auditory receptive fields of the neurones in monkey superior colliculus² belie the precision with which the monkey can fixate an auditory 'target'. Precision within a motor map is more a matter of defining the boundaries of the motoneurone pool than of limiting the stimulus to a particular location, so one must take care to establish what the map is designed to represent.

Motor considerations can also help one to understand why the monkey's auditory map should move coordinately with the eyes, instead of having the head and ears as a fixed reference frame as one might expect of a map generated from head and ear cues. Apparently, what is being mapped onto the superior colliculus is the error between the current sound source direction and the current visual axis. Since the superior colliculus of the monkey has a motor map of saccadic eye movement vectors, the combination of the two maps would enable eye movements to be accurately programmed to fixate an invisible sound source. We can thus think of a map in 'motor-error space'.

A requirement for the motor-error schema is that a map has available a neural representation of current eye position. Such a representation also forms an important part of most successful models

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of saccade-generation, the most notable of which (Robinson's bang-bang model) uses the degree of mismatch between a current-eye-position signal and an intended-eye-position signal to provide the drive for saccadic burst generators¹². Just where the eye-position signals originate and how they are integrated with the map of auditory space remain difficult questions for the future, particularly in view of the current

lack of information about how the auditory space map is generated in the monkey's midbrain. Future work on the midbrain maps therefore promises to illuminate a variety of important issues concerning the sensory-motor interface. □

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Zoology

Back from the brink of extinction

from Jared M. Diamond

A RECURRING nightmare for conservation biologists is the discovery that the few surviving individuals of an endangered species are all of the same sex. In 1976 this nightmare came close to reality for the New Zealand Wildlife Service, with the discovery that of seven remaining Chatham Island Black Robins only two were females. This precipitated a drastic rescue programme, involving capture of the entire robin population, its transfer to another island and the fostering of eggs in the nests of two other species of song bird. As a result, for the first time in nearly a century, robins now occupy two islands¹. The significance of the rescue programme, however, goes far beyond the fate of one obscure species of bird because without such sophisticated management techniques a growing number of species would be doomed and yet the techniques arouse passionate opposition.

Why was the Black Robin (*Petroica traversi*) on the verge of extinction in 1976? By the 1870s the cats, rats and forest-clearing activities of European settlers had eliminated the robin from the larger of the Chatham Islands, 800 km east of New Zealand, restricting it to Mangere and Little Mangere. After cats were introduced to Mangere in the 1890s they proceeded to destroy its robins and 11 other bird species, leaving all of the world's Black Robins in 4 hectares of scrub on the windswept top of Little Mangere. By 1973 there were 17 adults and 1 juvenile^{2,3}.

On Little Mangere the robins' long-term prospects of survival seemed poor. Apart from the risks intrinsic in the island's tiny area, it was riddled with the nesting burrows of Sooty Shearwaters, its forest was dying and few young robins were surviving. After considering the options, the New Zealand Wildlife Service decided to plant 100,000 tree cuttings on Mangere Island itself (where the cats had died out), intending to transfer the robins there when the trees had become tall enough to provide suitable habitat. Meanwhile it began transfer experiments and detailed field studies with the Black Robin's closest relative, the New Zealand Robin (*Petroica australis*)³.

Unfortunately, the continued deterioration of Little Mangere's patch of scrub, and the poor breeding success of robins

there, made it impossible to wait. When the 1976 census revealed only two pairs and three bachelor males, making the Black Robin the world's rarest accurately censused bird, the Wildlife Service decided on a drastic risky measure: the immediate transfer of robins to the 4.2 hectares of existing scrub on Mangere.

Rough seas, bad weather and an almost fatal boat accident aborted the first two attempts⁴. But between 17 and 21 September 1976 both pairs and one bachelor were caught and transferred and at the end of the breeding season, in March 1977, the two remaining males joined them on Mangere⁵ so that the whole population of the Black Robin was on the one island.

Although the birds immediately began to breed on Mangere, recovery of the Black Robin population was slow because only two eggs are laid, and at best only one of those is likely to yield a fledged young. However, like many bird species, Black Robins will lay a second or even a third clutch if the previous clutch fails. So, in 1980 the first clutches of the two surviving pairs were transferred to nearby nests of another small insectivorous bird species, the Chatham Island Warbler. The robins relaid, and four robin chicks were fledged that year — three of them by warblers. But the warblers were incapable of caring for the growing robin chicks for more than ten days, after which they had to be fostered back to adult robins. So, in 1981 the first clutches were transported to nearby South East Island for foster-rearing by the Tomtit (*Petroica macrocephala*). These proved ideal foster parents and raised three of the five robin chicks produced that year¹.

To reduce the risk of inbreeding or of extinction by a local disaster on Mangere, where the robin habitat is at the base of an unstable cliff, two pairs of adult robins were transferred to South East Island in 1983. Despite unusually bad weather leading to the deaths of four robins in 1982 and 1983, the species entered the 1983–84 breeding season with three breeding pairs.

These interventions obviously involved big risks, both to the Black Robin and to the New Zealand Wildlife Service itself. Even before the near-fatal boat accident in 1976, the Service was aware that public

opinion would tolerate the loss of a ranger during the transfers, but not of either of the two surviving female robins². The courage and experience required to move an entire population of an endangered species grew out of need. Faced with destructive introduced mammals on the New Zealand mainland, inadequate budgets and the responsibility for an unusually high proportion of endangered or vulnerable endemic species, the New Zealand Wildlife Service has developed management techniques based on detailed studies of the ecology and sociobiology of endangered species. Foremost among them has been the introduction of threatened taxa to offshore islands from which introduced mammals are absent or have been eradicated. Such taxa as the South Island Saddleback, Buff Weka and Little Spotted Kiwi would be extinct today were it not for such introductions.

Nevertheless, in New Zealand as elsewhere, the philosophy of drastic intervention to save endangered species elicits opposition as well as support, even among biologists. The debate over whether to transfer some Black Robins from Mangere to South East Island is not unlike the debate in the United States over the captive breeding programme for the California Condor. Sometimes opposition is based on doubts about the particular form of intervention proposed, the risk of individuals being killed and the possibility that a programme will draw off too much money from other conservation programmes. Partly, too, the opposition stems from revulsion at intense artificial manipulation of wild populations, even in the hope of saving them: should not the last condors be allowed to die in dignity in the wild, rather than as captives in a zoo? Breeding of Black Robins will have to be managed for a while to avoid effects of inbreeding, and perhaps to avoid imprinting on foster-parents. Does this not blur the distinction between maintenance in the wild and in a zoo, and thereby virtually convert Mangere and South East Island into outdoor zoos?

Many people, however, feel that managed robins are better than no robins and that humans should devote ingenuity and massive intervention to saving species as well as to exterminating them. Without intervention in 1976 the Black Robin would soon have met the fate of the Dusky Seaside Sparrow. For this doomed bird of Florida marshes, the conservationists' nightmare has come true: the few surviving individuals are all males. □

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Protein crystallography

Fifty years of pepsin crystals

from Guy Dodson and Cyrus Chothia

IN the middle of April 1934, J.D. Bernal and Dorothy Crowfoot (now Hodgkin) obtained the first successful X-ray photographs of a globular protein. They were obtained in Cambridge from crystals of pepsin grown by John Philpot in Uppsala. The photographs, discussed in a letter to *Nature* (133, 794; 1934) published half a century ago, almost to the day, were the start of one of the great achievements of modern biology.

On 13 April 1984 a meeting was held in Cambridge to commemorate the fiftieth anniversary of the experiment. Lectures were given by Dorothy Hodgkin, Max Perutz and Aaron Klug, all of whom worked at different times in Bernal's laboratory. The occasion brought together all those still living who were directly involved in the pepsin experiment; sadly, Peter Wooster, a long-established figure in Bernal's laboratory and remembered warmly by many, died on the night preceding the meeting. Several crystallographers who had contributed to the field, notably Arthur Wilson (intensity statistics) and Henry Lipson (Fourier calculation methods), were also present.

Part of the meeting was devoted to a recreation of the events that led to the pepsin crystal's coming to Bernal's laboratory at Cambridge and to the crystallographic examination of pepsin and other protein crystals (including viruses) that subsequently took place. The historical accounts were filled out, sometimes straightened out, particularly by John Philpot on the background of the crystallization of pepsin (in explaining why he went to Uppsala to grow the crystals he distinguished nicely between his real reason for going there — a young lady — and his excuse — to work on the ultracentrifuge) and by Bill Pirie on early virus experiments. The history of pepsin was completed by a comment from Tom Blundell on the structure of the pepsin molecule inferred from homologous enzymes.

The impressions that emerged of Bernal's laboratory were of great activity and many abilities, and of Bernal's omnivorous scientific interests. The laboratory already had a famous reputation, particularly for the work on sterols, and it was this, or Dorothy Crowfoot's reputation, that apparently prompted Glen Millikan, a peripatetic scientist, to bring John Philpot's crystals to Cambridge.

When the crystals arrived, Bernal immediately examined them in a polarizing microscope (Dorothy Crowfoot was away with what proved to be an incipient rheumatic attack). He found them to be beautifully formed, showing sharp birefringence while in the tube they had been grown in.

The crystals failed to diffract after removal from the test tube and drying but, crucially, Bernal noticed that their birefringence had also disappeared. Thus he realized the need to keep crystals wet. Bernal's solution was to put the crystals in capillaries (made possible because of Helen Megaw's studies on ice crystals). The subsequent investigation of the crystal's diffraction characteristics, cell dimensions and space group was carried out by Bernal and Dorothy Crowfoot. It led to their recognition "that the arrangement of atoms inside the protein molecule is of a perfectly definite kind" and to remarkably accurate ideas about the molecules' molecular weight and shape.

The descriptions of the subsequent research into protein crystals included the details of how the studies on insulin, haemoglobin and the viruses began. The immense difficulties in solving these structures were clearly appreciated at the time and the early investigations were devoted to measuring X-ray intensities, to calculating the Patterson function (also celebrating its fiftieth anniversary this year) and to characterizing new protein crystals. The influence of Bernal and Bragg was clearly crucial. A typical contribution was Bernal's letter to Dorothy Crowfoot suggesting that the isomorphous replacement of Zn by Cd in the rhombohedral insulin crystal could give phase information. Bragg's role, more concentrated at first on haemoglobin and later on myoglobin, was quieter but his steady sympathy, persistence and deep crystallographic insight helped the early research through disappointing years. Perutz recalled Bragg's grasp of crystallographic principles and illustrated it by the studies on how the shrinkage of protein crystal cells could be used to define centric phases.

In his review of virus research and the structure of water, both deep interests of

Bernal, Klug emphasized the importance of flexible thinking and how Bernal's aphorism, 'crystallographers must learn not to be crystallographers', applied over and over again. This was as true in Pauling's production of the α -helix structure and in Bernal's approach to the water problem as it is now in the study of the behaviour of protein structure which is increasingly found to link well defined domains to flexible and variable segments. Thus, the linking of order and disorder to achieve assembly and function (RNA binding) in the tobacco mosaic virus is revealed by crystallographic analysis. A report by Steven Harrison (Harvard University) of the 2.8 Å resolution crystal structure of tomato bushy stunt virus nicely completed studies begun by Bernal and Fankuchen. This virus also demonstrates beautifully the order-disorder phenomenon, which Harrison showed is related to the packing and assembly of the virus subunits.

There are many conclusions from a day like this. First, there are the remarkably happy collaborative traditions in protein crystallography, established from the earliest days, that make it such fun to work in the field. Second, the techniques, essentially physics, have put the description of protein and biomolecular structure into the hands of crystallographers. Finally, the importance of crystals and other ordered systems as a route to the description of biochemical and biological structure remains paramount. Bernal saw these descriptions as the first stage — their analysis should lead to the principles which govern the assembly and integration of molecules into higher organizations. Already, as Richard Henderson emphasized, crystals of very large molecular assemblies such as the nucleosome have been solved. Thus, as long as there are crystals, the future of the field is assured and more than justifies the great excitement produced by those first X-ray photographs of pepsin. □

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Dorothy Hodgkin, John Philpot and Helen Megaw at the meeting. (Photograph by Claudio Villa.)

Crystallography

Defects in reduced oxides

from C.J. Humphreys

CRYSTALS are like people: it is the defects in them which tend to make them interesting. In recent years, solid-state physicists and chemists have become increasingly aware that real crystals are usually not perfectly periodic arrays of atoms. Instead they contain a variety of isolated defects, which are important because they can strongly influence the physical and chemical properties of the crystals. An article on page 319 of this issue, by Bursill and Smith, reports the use of high-resolution electron microscopy to provide new information on the isolated defects which form when oxide crystals are reduced. This is the latest development in understanding reduced oxides, which have long puzzled chemists and physicists.

The basic question is the following: when oxide crystals are reduced (for example, $\text{MoO}_3 \rightarrow \text{MoO}_{3-x}$), are the resulting oxygen vacancies in the structure distributed homogeneously or are there isolated extended defects, and if so how do they form? Until 1950 it was believed that the oxygen vacancies were distributed homogeneously. Then A. Magnéli (*Ark. Kemi* 1, 513; 1950) observed extra diffraction spots in X-ray diffraction patterns from reduced oxides which he correctly attributed to an ordered array of defects called crystallographic shear planes (CSPs). Five years later A. D. Wadsley postulated the existence of isolated CSPs (*Rev. pure appl. Chem.* 5, 165; 1955). But the then available techniques of X-ray and neutron diffraction gave diffraction patterns which yielded atomic positions statistically averaged over a relatively large diffracting volume (typically 1 mm^3) of the crystal and so were incapable of detecting individual crystal defects. It took another fourteen years before Wadsley's predictions were confirmed by the observation of isolated CSPs by electron microscopy (Bursill, L.A. & Hyde, B.G. *Phil. Mag.* 20, 657; 1969).

We can think of CSP formation as follows. If it is energetically favourable for them to do so, oxygen vacancies will cluster together on a crystal plane rather than exist as isolated vacancies. The crystal on each side of this cluster of vacancies will then collapse into the missing layer of oxygen atoms and also shear sideways to reform chemical bonds. The defect so formed is a CSP. In MoO_3 the local chemical composition at a CSP is MoO_2 . Hence a reduced crystal of overall composition MoO_{3-x} can be thought of as a matrix of MoO_3 plus the appropriate number of CSPs of composition MoO_2 . We thus have a beautifully simple concept of how oxygen vacancies are accommodated in reduced oxides. If there are a number of CSPs in a crystal they

will form an ordered array at a sufficiently high temperature, as Magnéli originally observed.

The picture I have just given is a simplified one. In particular, for a very small degree of reduction (corresponding to $x < 0.005$) CSPs are not usually observed. Are the oxygen vacancies at these low concentrations uniformly distributed or are they concentrated into isolated small defects, and how do the CSPs form and grow? These are the questions that Bursill and Smith can address in their paper

because of the availability of the latest generation of very high-resolution electron microscopes, with a resolution of less than 0.2 nm. They show, for the first time, that there are small localized defects which precede the CSPs and that these defects may be characterized through careful analysis of very high-resolution electron micrographs. Thus we gain new insight into the earliest stages of structural changes that accompany reduction. This work is not only important for chemists and physicists studying oxides but also illustrates the power of high-resolution electron microscopy for studying, at the atomic level, the structure of defects in a wide range of materials. □

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Immunology

More on the T-cell receptor

from Philippa Marrack

NOT surprisingly, one of the main subjects of interest at a recent symposium* on 'Regulation of the immune system' was the receptor on T cells which allows them to respond clonally to antigen in association with products of the major histocompatibility complex (MHC). A year or two ago, when the molecules responsible for this feat were identified on the surfaces of T-cell clones, using clone-specific antibodies, they were shown to be disulphide-linked proteins made up of two different chains each of molecular weight 40,000–50,000 and containing variable and constant peptides. The T-cell receptor was therefore recognized to be strikingly similar in overall design to immunoglobulins. Recent papers in *Nature* (308, 145 & 149) reported the isolation of cDNA clones which probably encode one of the two polypeptide chains.

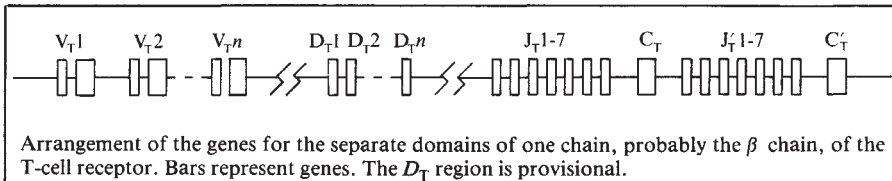
Since nearly all the protagonists involved in these discoveries were at the meeting, immunologists were given an opportunity to be brought up to date on all aspects of this work. Steven Hedrick (University of California, San Diego) and Tak Mak (Ontario Cancer Center) reviewed the data on the cDNA clones. The mRNAs from these clones are T-cell specific, come from genes which rearrange between the germ line and mature T cell, and are similar but not identical to immunoglobulins in nucleotide sequence. Moreover, the proteins they encode are very immunoglobulin-like in sequence and, in particular, include cysteines which could mark the domain-like structures so characteristic of this type of molecule. Hedrick also pointed out that the cysteine near the carboxy-terminal end of the protein is in a position analogous to the cysteine on immunoglo-

bulin light chains. Since the latter is involved in bridging light chains to heavy chains in most immunoglobulins, the former may have a similar function in the T-cell receptor, being used to bridge the two chains of the heterodimer.

Mark Davis and Nicholas Gascoigne (Stanford University) described the structure of mouse genomic clones encoding these sequences; their data are published on page 322 of this issue. Again there are striking similarities with immunoglobulin, since several segments of DNA, which occupy separate loci in the germ-line genome but are brought together by rearrangement in T cells, contribute to T-cell receptor cDNA (see the figure). One segment contains a cluster of variable-region (V_T) genes, mapping an undetermined distance from a proposed segment containing several diversity-region (D_T) genes (DNA encoding this segment has not yet been found). A third segment contains a cluster of seven joining-region (J_T) genes and a constant-region (C_T) gene. The last contains four exons, of which three were expected: the exons for the constant-region immunoglobulin-like domain, which is extracellular, a transmembrane sequence, and a cytoplasmic domain. But the fourth, encoding four to six amino acids between the extracellular domain and the transmembrane sequence, came as a surprise. This extra domain has some sequence homology with the hinge region of immunoglobulins, the part thought to confer flexibility on antibodies. A lack of prolines in the hinge region of the T-cell receptor suggests it might not be quite so flexible. The cysteine equivalent to that thought to be involved in interchain disulphide bonds in antibody molecules is found in this exon.

Davis and his co-workers have found

*The Ortho-UCLA symposium on 'Regulation of the immune system' was held in Park City, Utah, 18–25 March.



two very similar C_T genes, close together on the same genomic clone. Each seems to have its own cluster of J_T genes; there is evidence that each V_T gene can feed off either cluster of J_T s. If so, this arrangement would be similar to that of the immunoglobulin light chain, λ . These results were confirmed by Mitch Kronenberg (Caltech). The human gene structure seems to be similar since Mak has also found two closely arranged constant-region genes. In mouse the genes map to the proximal region of chromosome 6 (Davis and Kronenberg).

There is some evidence that the T-cell repertoire, at least reflected by use of these genes, is limited. For example, there is no evidence for somatic variation, although so far only a limited number of sequences have been looked at. Davis mentioned that the V_T -region sequence found in a T-cell hybridoma specific for pigeon cytochrome *c* in association with $I-A^k$ is identical to its germ-line counterpart. Perhaps less surprisingly, Hedrick showed that three independent T-cell hybridomas with the same specificity for cytochrome *c* and $I-A$ seem to use the same V_T region, at least for this chain, since all show the same size rearrangement of the gene.

Which of the two polypeptides, unimaginatively called α and β , of the T-cell receptor are coded for by the genes detected by the cDNA probes? Ellis Reinherz (Harvard University) and his collaborators have shown that the amino-terminal sequence of the variable region described by Mak is almost identical to the amino-terminal amino acid sequence they have obtained for the β chain.

With every indication that the arrangement and rearrangements of T-cell receptor genes have been, or soon will be, worked out, interest turned to their expression. All investigators agreed that the genes are rearranged and transcribed only in T cells although not, perhaps, in suppressor T cells. The high level of mRNA produced from the genes in thymocytes is of particular interest because the thymus is the organ in which the ability of T cells to recognize antigen in the context of self-MHC is thought to be selected. Presumably this selection depends on the expression of receptor proteins on thymocyte surfaces. Some doubt that high levels of mRNA necessarily result in high levels of cell-surface receptor was raised by John Kappler (National Jewish Hospital, Denver), who described a monoclonal antibody which binds to the receptors on about 20 per cent of peripheral T cells in most mouse strains. Each of these cells has $3-4 \times 10^4$ receptors on its surface whereas

the number of receptors on immature thymocytes ranges from zero to about the same as on the peripheral T cells. Those without any receptors may not yet have generated the ability to synthesize mRNA for both chains of the receptor, or may have lost this ability because of mutation or aberrant rearrangement.

Since cytotoxic T cells usually recognize antigen in association with class I products of the MHC, whereas helper T cells usually recognize antigen in association with class II MHC products, some difference between the receptors on the two types of cells has long been expected. Although no differences were identified with the cDNA probes or with Kappler's antibody, a first inkling of their existence was suggested by J.P. Allison (University of Texas Cancer Center, Smithville) who showed that antibodies produced in rabbits against the purified receptor from a mouse T-cell leukaemia preferentially react with a subset of receptors on L3T4-bearing mouse T cells, the cells thought to be mainly class II-restricted helper T cells.

Some years ago Jerne suggested that an immunological network controls the specificities of lymphocytes in any given animal since each new variable-region sequence is, in a sense, an antigen new to the animal and so potentially recognizable by other variable regions — of antibodies or on T cells. Hence the suggestion that there might be a subset of T cells which recognizes the T-cell receptor on other T cells and does so by means of a receptor that bears some resemblance in structure to

the MHC antigens recognized by the T-cell receptor. Sim and Augustin (Denver) supported that suggestion with evidence that a T-cell hybridoma, prepared from mice immunized with an anti- $I-A$ antibody, responds to that antibody in the absence of any other stimulus, and that the antibody can precipitate from the hybridoma a structure similar to other antigen-specific MHC-restricted T-cell receptors.

The idea that T cells might bind anti-MHC antibodies, in particular those directed against class II-like molecules, not because they bear products of the MHC itself but because they bear receptors which resemble MHC products (internal images), was used at several points in the meeting to explain the still mysterious existence of antigens on T cells which seem to map genetically in the *I* region, especially the *I-J* region, of the MHC. But why, then, are *I*-region-related messages not expressed in T cells (Kronenberg)? Colleen Hayes (University of Wisconsin) and her colleagues have recently published data which suggest that the determinants found on T cells known as *I-J* are controlled in the mouse by genetic loci mapping both in the MHC, on chromosome 17 and on chromosome 4, suggesting some sort of complementation (*Science* 223, 559; 1984). Internal-image devotees now theorize that *I-J*, and other structures on T cells which react with anti-MHC antibodies, may be T-cell receptors which mimic the MHC but which are encoded by genes not mapping at this locus. This may be true for MHC-restricted helper and cytotoxic T cells, as exemplified by the Sim and Augustin experiment, and for free-antigen-binding suppressor T cells, which apparently have a different type of receptor. □

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100 years ago

NOTES

M. PASTEUR read to the Academy of Sciences on Monday an account of his experiments on rabies. He maintains that he has twenty dogs which he has rendered insusceptible to the disease, and which, with twenty ordinary dogs, he is prepared to have bitten by a number of dogs in a rabid state. A Commission has been appointed by the French Government to test M. Pasteur's conclusions, the immense importance of which, if established, must be evident to every one. Eminent physiologists maintain, however, that M. Pasteur is far from having proved his position, and that it would be rash to give any positive opinion upon the subject until the experiment which he suggests has been made. We await the full report of M. Pasteur's paper before saying more upon it.

From *Nature* 30, 22 & 29 May 1884.

A REMARKABLY BRILLIANT METEOR

TO-NIGHT, about 10.45 p.m., I was "stepping westward," about half a mile east of my house. Suddenly the ground before me was lighted up with moonlike splendour by a luminary that was above me and behind me. Looking back I saw a meteor a good deal east of the Grati Bear, and nearly as high in the sky. It was about as big as Venus, and of the same hue. It was speeding from north to south with a slight descent. Its course very soon came to an end. It left behind it a streak of duller lustre: this phosphorus-like trail vanished almost at once. The career of this meteor while that body was visible here, lasted little, if at all, longer than a minute, but its light was remarkably brilliant.

DARK TRANSIT OF SATELLITE

ON May 18, at 8 h., on observing Jupiter with my 10-inch reflector, p. 252, I saw three very dark spots. These I took to be the shadows of satellites, and on reference found that the shadows of Satellites I. and II. were really upon the planet; also Satellite I itself. I believe the very dark appearance of the satellite on this occasion to have been somewhat exceptional; for though I have observed a considerable number of its transits, I never saw it nearly so dark before.

Molecular biology

DNA bending and kinking

from Jonathan Widom

To pack double-stranded DNA into the protein shell of viruses such as phage λ , or to wrap it around the protein cores of the nucleosomes that characterize the chromatin of higher organisms, requires the DNA to be tightly bent or kinked at many sites. In the simplest case, DNA may have only a passive role in its packaging. In other cases, sequences within the DNA may specify the bending or kinking, or bends or kinks could be induced by the binding of proteins. Experiments described in a recent issue of *Nature*¹ and on page 327 of this issue² provide evidence of both of the active mechanisms of bending or kinking.

To understand the degree of bending required of DNA packaged in viruses and in chromatin, one must have a quantitative measure of the stiffness of isolated DNA. This has been obtained by hydrodynamic methods³, and extended to tight bending (~ 240 base pair circles) by Shore *et al.* using an enzymatic ring-closure method⁴. The persistence length of random-sequence DNA is found to be ~ 500 Å, or ~ 150 base pairs (bp)³. DNA must be tightly bent or kinked when packaged in phage λ because the hollow protein capsid has a diameter of only 600 Å (ref.5). On a nucleosome, DNA is bent through 360° in the space of ~ 80 bp (ref.6), only half of the persistence length of DNA in solution.

Adding to the interest in what determines bends or kinks in DNA is the possibility that altered DNA conformation may serve as an important signal in the sequence-specific binding of proteins. Thus in 1975, Crick and Klug⁷ pointed out that several base pairs at a kink may be particularly accessible to proteins and, on the basis of model-building studies, several groups have proposed non-B-form conformations for DNA bound to proteins (for example, refs 8 and 9). Although the structures of three sequence-specific DNA-binding proteins (cro¹⁰, cI¹¹, CAP⁹) have been solved by X-ray diffraction, the proteins were not bound to DNA, so only by model-building could their mode of binding be tentatively predicted. That is the reason for the special importance of the two new papers^{1,2} which clearly show striking departures from linear B-DNA in both isolated DNA and in DNA-protein complexes.

Wu and Crothers report an elegant experiment which indicates that a particular DNA sequence may cause the DNA molecule to bend¹. The DNA they have worked with is a restriction fragment from the kinetoplast of a trypanosome. The kinetoplast contains a large number of highly-interlocked, very small (~ 800 bp) DNA circles of unknown function. Marini *et al.* had reported anomalous electro-

phoretic and hydrodynamic behaviour for one particular restriction fragment, which led them to postulate that the fragment may be bent¹². To test that suggestion, Wu and Crothers devised an elegant technique (circular permutation) of generating from the anomalous restriction fragment a series of variants differing only in the position of the suggested bending locus relative to the ends of the fragment. When these essentially identical restriction fragments were run on non-denaturing acrylamide gels, a striking range of mobilities was observed, consistent with the proposal that the original fragment was bent at a specific site. Theories of gel electrophoresis¹³ predict (for a given molecular mass) a dependence of mobility on h^2 , the mean-squared end-to-end distance of the molecule. If a sequence at the centre of a restriction fragment causes that molecule to bend, it will have a reduced h^2 and hence should migrate more slowly in gels. If that same restriction fragment is circularly permuted to place the site of bending at the very end of the molecule, h^2 should be restored to its expected value, so the fragment should migrate more rapidly. By extrapolating their mobility data, Wu and Crothers are able to pinpoint the DNA sequence that causes the bend. In the restriction fragment from the kinetoplast minicircle (K-DNA), they find a very striking sequence at the centre of the bend: the sequence C(A)_{5,6}T is repeated four times, separated by three or four other bases so that the sequences are spaced ~ 10 bp apart, in phase with the twist of the DNA helix.

Theories of gel electrophoresis are not sufficiently developed to allow one to be certain of the reason for the anomalous mobility of K-DNA. An alternative explanation, not considered by Wu and Crothers, is that rather than conferring a permanent bend with definite geometry, the sequence C(A)_{5,6}T could simply confer increased flexibility, and that increased flexibility results in slower migration in gels. Hogan *et al.*¹⁴ recently reported enhanced flexibility for the sequence poly(dA)·poly(dT), and Charney and colleagues (personal communication) have found a greatly enhanced flexibility for the sequence poly(dA-dT)·poly(dA-dT).

Although it is therefore reasonable to expect that the sequence found by Wu and Crothers will have an increased flexibility, two lines of reasoning suggest that increased flexibility alone cannot account for their results. First, Charney and colleagues find that, while having substantially enhanced flexibility, poly(dA-dT)·poly(dA-dT) does not behave anomalously on polyacrylamide gels. Second, increased flexibility would not account for the

striking 5 and 10 bp periodicities of the K-DNA bend locus. Wu and Crothers suggest that runs of dA_{5,6} may have an altered helical conformation, such as the heteronomous DNA (H-form) postulated by Arnott and colleagues¹⁵. When a B-form to H-form junction is created, it is likely to involve a bend¹⁵. The two strands of H-form DNA are non-equivalent, so there will be a directionality to an H-B junction (with respect to the polar phosphate-sugar backbone), producing bends of opposite sense at H $\rightarrow$ B and B $\rightarrow$ H junctions. Because the runs of A are 5–6 bp long — half of the helical periodicity of DNA — bends of opposite sense at the entrance and exit of A_{5,6} blocks will point in the same direction to produce a planar bend rather than a zigzag. There are four of these units in the K-DNA bending locus; because the units themselves repeat every ~ 10 bp, planar bends from individual units add in-phase to produce a large net planar bend. If these sequences promoted bending merely by virtue of their flexibility, the 5 bp periodicity, especially, would be unnecessary and not expected.

In a parallel series of experiments, Wu and Crothers showed that the mobility of circularly permuted variants of a restriction fragment from the *lac* control region of *Escherichia coli* does not vary with circular permutation, even when *lac* repressor tetramers were bound at the operator site. When dimers of CAP protein (which are much smaller than *lac* repressor tetramers) were added, however, there was a marked dependence of the mobility on the degree of circular permutation. Previous experiments have shown that both CAP and *lac* repressor remain correctly bound to the DNA during electrophoresis (see refs 1, 16). Wu and Crothers conclude that the *lac* DNA fragment is itself unbent but that it bends in response to the binding of CAP protein. The same conclusion based on related experiments had previously been reached by Kolb *et al.*, who obtained fragments with the CAP-binding site at different locations relative to the fragment ends from many different control regions of *E. coli*¹⁶. By extrapolating their mobility data, Wu and Crothers map the CAP-induced bend to a point within the CAP-binding site that is several bases towards the promoter from the binding site centre of symmetry. This raises the possibility that CAP acts to promote transcription by stabilizing an altered DNA conformation which may be favoured by RNA polymerase.

Turning to the paper of Frederick *et al.*, on page 327, one is presented with the striking results of the first X-ray diffraction structure of a complex of protein and DNA²; the results bear directly on the issues of sequence-specific recognition and DNA packaging. Frederick *et al.* have studied the complex of the restriction enzyme *EcoRI* with DNA, formed in the absence of Mg²⁺ so that the enzyme did not hydrolyse the DNA. The DNA has the

sequence: TCGCGAATTCGCG
GCGCTTAAGCGCT

That sequence contains the recognition sequence for *EcoRI*, GAATTC, and is closely related to the dodecamer studied by Dickerson and colleagues¹⁷. Frederick *et al.* find that the protein-bound DNA has several unusual features, including three kinks. One kink (the 'neo-1' kink) is in the centre of the DNA molecule, on the dyad symmetry axis, and represents a torsional dislocation between two segments of B-form DNA. One can think of this kink as being generated in two steps: first, the DNA is unwound by 25° between the base pairs to either side of the kink, then the DNA is bent 12° into the minor groove. This is the mechanism of kinking postulated by Crick and Klug⁷ in their kinked-helix model, but the present neo-1 kink differs from the Crick-Klug kink by restricting itself to a modest 12° bend, rather than taking advantage of the full 100° bend that model-building suggests is available. In the complex with *EcoRI*, the neo-1 kink is required to widen the major groove, facilitating access to the bases and allowing an extensive complementary interface with the protein dimer.

The other two kinks ('neo-2 kinks') differ from the neo-1 kink, but are identical to each other because they occupy equivalent positions about the complex's dyad axis. The neo-2 kinks separate the terminal (T)CGC blocks from the central GAA blocks and are produced in a different way from the neo-1 kink. They lack noticeable unwinding; a base-roll¹⁷ into the minor groove produces a bend of 23°. While the central GAA blocks appear to be B-like, the terminal (T)CGC blocks appear to be A-like. Frederick *et al.* suggest that the neo-2 kink may arise from the joining of these two different DNA conformational blocks. It should be noted that in a previous model-building study, Selsing *et al.*¹⁸ found that they could not build a stereochemically acceptable A-B junction of the sort proposed here. Selsing *et al.* found it necessary to have at least one intervening base pair separating orthodox A- and B-form regions. A better understanding of the neo-2 kink must await determination of the atomic positions of the base pairs involved.

A final striking feature of the *EcoRI*-DNA complex concerns the effects of DNA sequences flanking the enzyme's recognition sequence, GAATTC. Different flanking sequences are known to influence the hydrolysis rate by an order of magnitude¹⁹. Surprisingly, therefore, Frederick *et al.* see no contacts between *EcoRI* and DNA bases outside the recognition sequence; only the backbone appears to be contacted. They conclude that the flanking sequences may affect enzyme activity by changing the conformational free energy of the neo-2 kink or of the GCC terminal blocks. An alternative possibility is that different flanking sequences may give rise to different local

DNA backbone conformations, for which the enzyme may have different affinities.

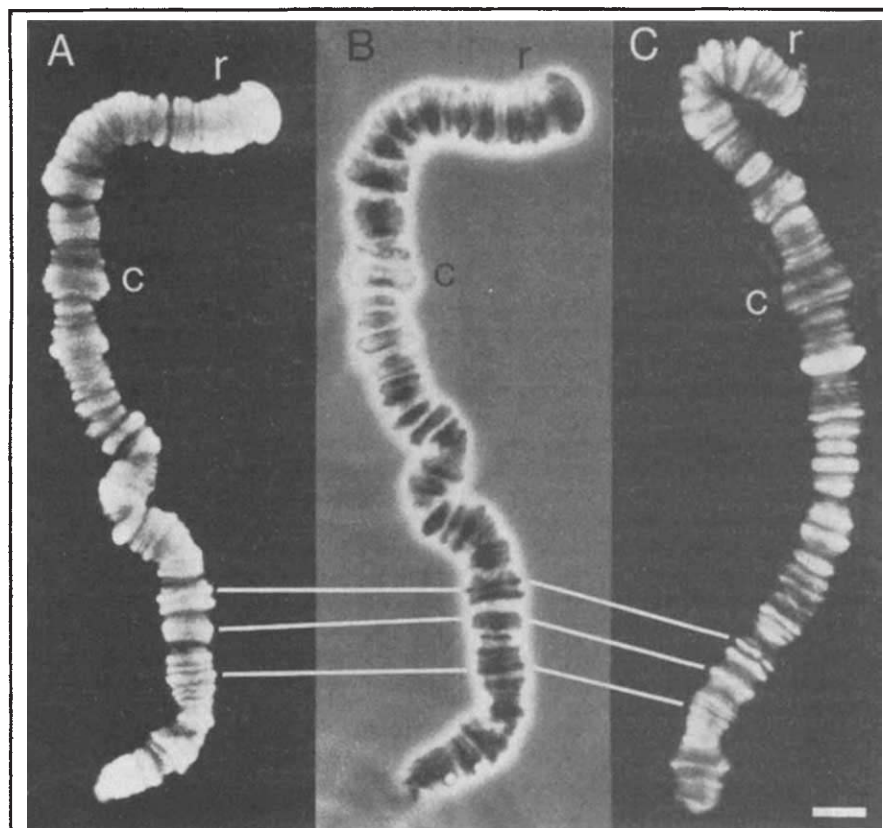
One must be aware that *EcoRI* is a hydrolytic enzyme, whose function is to cut the DNA chain between the bases G and A in the sequence GAATTC. The site of cleavage is only 1 bp away from the neo-2 kink and 2 bp away from the neo-1 kink. Presumably the enzyme acts by stabilizing a transition state in which the G-A backbone has an increased lability to hydrolysis. It is feasible that either the neo-2 kink or the neo-1 kink may destabilize the G-A backbone bond, and indeed this may turn out to be the *raison d'être* of the neo-2 kink. This does not detract from the important conclusion that for both CAP and *EcoRI*, and probably many other proteins, dramatically altered DNA structures will be important features in the DNA-protein complexes.

Taken together, the new studies of K-DNA and of CAP-DNA and *EcoRI*-DNA complexes show that the structure and function of a DNA molecule can be affected in important and unexpected ways both by the DNA sequence itself and by the

interaction of specific proteins with the DNA. These results suggest that altered DNA conformations are likely to play an important part in DNA packaging and in the sequence-specific recognition of DNA by proteins. □

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Z-DNA: the acid test

ACID treatment of polytene chromosomes of *Drosophila* before their exposure to an antibody against Z-DNA resulted in these striking immunofluorescent micrographs. A phase-contrast image of an isolated chromosome (B) allows its bands to be clearly identified and makes obvious that Z-DNA is associated with them, both in an acid-treated isolated chromosome (A) and in a 'squash preparation' of a chromosome prepared after acid fixation of the salivary gland (C). In less acid conditions, far less Z-DNA is apparent and it is confined to the periphery of the bands; acid treatment, by stripping proteins from the DNA and by protonating its bases, may both expose masked Z-DNA and induce the transition of B-DNA to Z-DNA. The lines connect bands, 'c' denotes centromere, 'r' the right-hand end of the chromosomes and the bar represents 10 µm. From Robert-Nicoud, M., *et al.* *EMBO J.* **3**, 721 (1984).

Mathematics

Beyond reasonable doubt?

from Ian Stewart

WHEREAS the mathematical discipline of Number Theory deals with properties of whole numbers, that of Analysis is concerned with the continuous properties of real and complex numbers. Despite this difference, Bernhard Riemann, in 1859, discovered profound connections between the two, leading to what is now known as Analytic Number Theory. Riemann developed a theory of the zeta function, defined for real $s > 1$ by

$$\zeta(s) = 1^{-s} + 2^{-s} + 3^{-s} + \dots$$

and for complex s by analytical continuation, and showed how it could be used to study the distribution of prime numbers (*Monatsber. Akad. Berlin*, 671; 1859). In particular, the number of primes in a given interval could be estimated provided enough was known about the zeros of the zeta function: values s such that $\zeta(s) = 0$. There are some relatively obvious zeros at $s = -2, -4, -6, \dots$. Riemann conjectured that all the remaining zeros lie on the complex line $s = \frac{1}{2} + iy$ where y is real. This, the Riemann Hypothesis, if true, has extensive repercussions — not all within Number Theory. It remains an intri-

guing and important unsolved problem.

Computer calculations, based on various known features of the zeta function, have shown that Riemann's hypothesis holds good for the first 320 million zeros (Edwards, H.M. *Riemann's Zeta Function*, Academic, New York; 1974). This would appear to be strong evidence in favour of the hypothesis — but is it? An analogous question is illuminating. In 1885 T.J. Stieltjes came up with a different conjecture which would, if true, imply the Riemann Hypothesis (*C. r. hebdom. Seanc. Acad. Sci., Paris* 101, 368). It was proposed again by F. Mertens in 1897, after whom it is now named (*Sber. preuss. Akad. Wiss.* 106, 761). It can be stated as follows. For any integer n , let $M(n)$ be the difference between the number of integers less than n that are products of an even number of distinct primes and the number that are products of an odd number of primes. The Mertens Conjecture is that $M(n) < \sqrt{n}$ for $n > 1$. For example when $\sqrt{n} = 16$ there are five numbers in the 'even' case (1, 6, 10, 14, 15) and six 'odd' (2, 3, 5, 7, 11, 13), so $M(16) = 1$ which is cer-

tainly less than 4. The Mertens Conjecture has been verified by computer calculations for the first 10 billion values of n .

Despite the apparently overwhelming evidence in its favour, it has recently been discovered that the Mertens Conjecture is false. Andrew Odlyzko (Bell Laboratories) and Herman te Riele (Centre for Mathematics and Computer Science, Amsterdam) have shown in unpublished work, based upon fast computer techniques, that there exist infinitely many values of n for which the conjecture fails. The first 'bad' n is no larger than 10 to the power 10 to the power 70 (or thereabouts), an order of magnitude totally inaccessible to direct computation. It is possible that an explicit calculation of the actual 'bad' values would not be an improvement on the demonstration that they must exist.

The Riemann Hypothesis is unaffected by this result: it is still unsolved. Experts have long felt that to prove it by way of the Mertens Conjecture would be 'too easy', and their suspicions are now vindicated. Meanwhile, if the first 10 billion numbers are conspiring to mislead us about the Mertens Conjecture, what faith can we place on a mere 320 million in favour of the Riemann Hypothesis? □

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Astronomy

IRAS circular 11

THE sources in this circular all have a firm detection at $60\mu\text{m}$, are at least 20° away from the galactic plane and have continua representative of 'warm' galaxies. Each search box has been inspected on SERC/ESO Schmidt survey plates or on National Geographic/Palomar Schmidt plates, and contains an obvious candidate, in

most cases a galaxy. Selection of sources and the optical search were done by G.K. Miley and M.H.K. de Grijp, Sterrewacht Leiden, The Netherlands. Source names derived as before; see *Nature* 309, 480; 1983. Position is given at equinox 1950.0. Measurements were made between epochs 1983.1 and 1983.9. In the table,

a m denotes arc min.

Also now available, but not to be published in *Nature* is IRAS circular 10 which lists 179 sources selected from the IRAS minisurvey of an area of approximately 300 square degrees between 9 and 16 February 1983 (see Rowan-Robinson *et al. Astrophys. J. Lett.* 278, L7; 1984). The sources satisfied the criteria of being more than 20° away from the galactic plane, reproducible on successive scans and with a signal-to-noise ratio of greater than 9:1 in at least one of the wavelength bands.

Source IRAS	RA h min s	Dec deg a m	Flux density (Jy) 12 μm 25 μm 60 μm 100 μm	Source IRAS	RA h min s	Dec deg a m	Flux density (Jy) 12 μm 25 μm 60 μm 100 μm
0425-072P11	04 25 22.2	-07 15	<0.4 0.4 0.9 1.3	1320-342P11	13 20 44.8	-34 15	<0.4 <0.3 0.6 <1.7
0425-046P11	04 25 57.1	-04 40	<0.2 1.6 4.5 4.3	1329+022P11	13 29 19.7	+02 16	<0.2 <0.6 1.1 1.2
0428-097P11	04 28 11.0	-09 44	<0.3 0.4 0.7 <1.4	1331-301P11	13 31 28.9	-30 07	<0.2 <0.3 0.8 <1.0
0432-143P11	04 32 32.5	-14 19	1.2 3.8 7.9 11.3	1331-234P11	13 31 51.2	-23 25	<0.2 <0.4 1.0 2.2
0438-084P11	04 38 28.8	-08 28	0.5 1.8 3.4 2.8	1331-231P11	13 31 56.4	-23 11	<0.9 <0.5 1.0 2.0
0450-032P11	04 50 14.1	-03 17	<0.6 0.5 1.0 1.5	1333-340P11	13 33 01.8	-34 02	0.4 0.7 1.2 1.5
0450-184P11	04 50 40.8	-18 26	<0.2 <0.5 0.9 2.5	1354-203P11	13 54 33.1	-20 22	<0.6 <0.8 1.5 2.5
0509-024P11	05 09 03.8	-02 26	0.3 1.2 2.0 <1.5	1356-188P11	13 56 16.2	-18 48	<0.3 <0.7 1.4 3.6
0513-235P11	05 13 44.2	-23 31	<0.2 <0.5 0.9 3.1	1402-316P11	14 02 09.7	-31 40	<0.5 <0.3 0.7 <1.5
0521-122P11	05 21 47.0	-12 12	<0.2 0.4 0.6 <2.8	1404+012P11	14 04 04.9	+01 17	<0.2 <0.5 1.1 <1.2
0531-206P11	05 31 57.0	-20 36	<0.2 <0.4 1.2 2.0	1423-116P11	14 23 27.8	-11 40	<0.4 <0.4 0.8 1.6
0556-348P11	05 56 31.9	-34 53	<0.7 <0.3 0.5 <1.3	1428-030P11	14 28 51.4	-03 04	<0.2 <0.4 1.0 2.0
0611-326P11	06 11 30.1	-32 40	<0.2 0.4 0.9 1.5	1431-326P11	14 31 42.8	-32 37	<0.3 0.4 1.0 <1.2
0815+035P11	08 15 18.0	+03 31	<0.4 <0.3 0.7 <1.7	1444-219P11	14 44 35.4	-21 56	<0.6 <0.4 1.1 1.8
0818+033P11	08 18 49.8	+03 19	<0.2 <0.4 0.8 2.0	1458-222P11	14 58 56.7	-22 15	<0.3 <0.4 0.8 2.6
1036-190P11	10 36 39.5	-19 04	<0.4 <0.3 0.6 <1.4	1509-211P11	15 09 06.6	-21 07	<0.4 0.7 1.8 2.0
1051-273P11	10 51 09.1	-27 22	<0.2 0.4 1.0 <1.2	1524+007P11	15 24 04.5	+00 46	<0.2 0.5 1.0 1.5
1105-115P11	11 05 48.9	-11 31	<0.2 0.4 0.8 <1.4	1548-037P11	15 48 03.4	-03 44	<0.4 0.8 1.3 <1.7
1119+045P11	11 19 55.6	+04 31	<0.7 0.5 0.9 2.7	1618+068P11	16 18 30.1	+06 51	<0.3 <0.3 0.7 <1.2
1121-281P11	11 21 33.3	-28 06	<0.4 0.4 0.7 <0.8	1832-594P11	18 32 32.8	-59 26	0.6 1.5 3.6 5.6
1246-111P11	12 46 53.3	-11 07	<0.2 0.8 1.7 2.1	1833-654P11	18 33 21.8	-65 28	0.8 2.5 2.6 <1.7
1249-131P11	12 49 35.1	-13 08	<0.2 <0.5 1.3 2.8	1840-624P11	18 40 07.9	-62 25	0.4 1.1 2.2 4.4
1304-234P11	13 04 23.5	-23 24	0.4 1.3 2.6 4.1	1844-532P11	18 44 14.7	-53 12	<0.2 <0.4 0.8 <1.7
1305-241P11	13 05 59.1	-24 07	<0.2 0.7 1.6 2.1	1919-421P11	19 19 23.9	-42 06	<0.4 <0.5 1.1 <2.1
1315-098P11	13 15 31.4	-09 49	<0.2 <0.5 0.9 <1.6	1955-140P11	19 55 49.9	-14 05	1.0 1.5 1.3 3.1
1316-242P11	13 16 49.3	-24 13	<0.2 <0.4 0.9 <2.1	1958-183P11	19 58 02.7	-18 18	<0.5 <0.7 1.1 1.4
1319-164P11	13 19 42.3	-16 27	0.8 2.9 6.3 7.1	2037-383P11	20 37 58.7	-38 22	<0.4 <0.5 1.5 <2.2

The IRAS fast-moving object search

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The Infrared Astronomical Satellite (IRAS) has provided an all-sky survey at wavelengths between 12 and 100 μm . The satellite also detected fast-moving objects in the Solar System, including new comets and Earth-crossing asteroids, one of which may be an extinct cometary nucleus.

THE first satellite dedicated to astronomical observations at IR wavelengths, the US/Netherlands/UK Infrared Astronomical Satellite (IRAS), was launched on 26 January 1983. The IRAS mission was intended to provide a highly reliable all-sky survey in four wavelength bands centred on 12, 24, 60 and 100 μm (refs 1–4) (These are usually described as bands I, II, III, IV respectively.) The satellite was also able to detect and identify fast-moving objects within the Solar System. In this paper we review the search procedures applied to IRAS data, and the consequent discovery of several new comets and asteroids.

Search strategy

The IRAS survey objectives of high reliability and completeness were achieved by repeated detections in each wavelength band. The detector layout in the focal plane, when combined with overlapping and repeated scans, allowed the positions of sources to be confirmed on time scales of seconds, hours and weeks. This process of repeated confirmation also provides a means of detecting moving objects, as sources which do not repeat at the same celestial coordinates are rejected by the processing software at the ground station. (It should be emphasized that the confirmation process is based on positional agreement, and not flux comparisons between detections; so fixed, but variable, sources are not rejected.)

The sources which were not confirmed during the survey may be divided into three groups.

- (1) Seconds confirmation failures. These include objects near to the spacecraft, such as Earth satellites, space debris and material evolved from IRAS; but most seconds confirmation failures are attributable to radiation hits on the detectors.
- (2) Hours confirmation failures. These include Solar System objects moving across the sky at more than $\sim 1 \text{ arc min h}^{-1}$ (for example, some comets, some main-belt asteroids and most Earth-crossing asteroids).
- (3) Weeks confirmation failures. These include slow-moving Solar System objects (such as distant asteroids, comets and planets).

Note that large numbers of detections in band II only, appeared to pass the seconds confirmation test. Examination of the raw data for these detections showed no evidence for the presence of a point source. These apparently spurious point sources should probably be attributed to fluctuations in the diffuse IR background. Chance pairings of these spurious sources were output as possible asteroid candidates. They represented by far the largest group of sources processed by the moving-object software and seriously hindered the search for faint asteroids.

Data from IRAS were transmitted to the Preliminary Analysis Facility (PAF) at the Rutherford Appleton Laboratory in the UK, where examination was confined to seconds and hours confirmation. The raw data were then transmitted to the Science Data Analysis System (SDAS) at the Jet Propulsion Laboratory, California for comprehensive analysis and production of the final data products. Slow-moving Solar System objects which

pass both seconds and hours confirmation will not be discovered until analysis is completed at SDAS. Owing to the unavoidable delay in carrying out this analysis, recovery and identification of the many new objects to be found will hardly be possible.

To exploit the potential of IRAS for detecting inner Solar System objects—especially those of low visible, but high IR brightness—appropriate software was developed. An object detected using this software may be described as a 'fast-moving object' (FMO). All sources which passed the seconds, but not the hours, confirmation tests were passed to this FMO detection software.

FMO detection software

The first selection process applied to seconds-confirmed, but hours-failed, sources was based on flux ratios. This was followed by a search for correlations between two comparable detections to provide information on the speed and direction of motion of the source.

The band I/band II and band II/band III flux ratios provide an indication of temperature, and were tested for all multiple-band, non-hours-confirmed detections. To allow for uncertainties in the PAF detector calibrations, these tests were set to pass all sources which gave indicated temperatures between 100 and 400 K. These limits were wide enough to ensure that no true inner Solar System objects were accidentally rejected. Due to source confusion near the galactic plane (which caused many fixed sources to fail hours confirmation), a galactic latitude limit was applied. Objects nearer to the galactic plane than $\pm 5^\circ$ were rejected.

One consequence of the hours-confirmation strategy (an overlap of half the array on successive orbits) is that a moving object will be observed as two separate non-hours-confirmed detections, if, after one orbit (100 min): (1) it moves more than the position errors due to detector size, and so on, involved in defining 'hours confirmation'; (2) it stays within the telescope field of view.

The minimum motion limit depends on the direction of motion and the number of bands in which the object is detected. In the in-scan direction (approximately along a line of ecliptic longitude), the detector width (for bands I and II) of 0.75 arc min and the satellite scan rate of $3.85 \text{ arc min s}^{-1}$ allow motions of $\sim 0.3 \text{ arc min}$ per orbit ($0.18 \text{ arc min h}^{-1}$) to be recognized. The cross-scan length of the band I and II detectors is 4.5 arc min, but improved resolution is obtained from the offset arrays⁴. Sources detected in more than one wavelength band therefore have more accurately defined positions: thus, smaller motions can be resolved for multiple-band sources. The smallest cross-scan motion which could be recognized was 1 arc min per orbit ($0.6 \text{ arc min h}^{-1}$).

The maximum motion which allows the object to remain in the 30-arc min field of view of the telescope depends critically on the component of motion in the cross-scan direction. Sources may have a cross-scan component of motion in the same direction as the scan advance (prograde), of up to 45 arc min per

Table 1 Alerts for which observations were attempted

Candidate	No. of detections	Date	RA	Dec	Magnitude	Observation
180-1	2	25 April	19h 06min	+49°	9	Comet IRAS-Araki-Alcock 1983d
214-1	2	13 May	09 17	-15	17	Comet IRAS 1983f
308-1	3	28 June	01 22	-22	15	Comet IRAS 1983j
315-2	2	2 July	11 03	-32	18	Possible main belt asteroid 1983 NH, lost
333-1	2	11 July	11 57	-49	18	Comet IRAS 1983k
334-1	2	11 July	04 13	-65		No object <18 mag
337-1	2	12 July	01 25	0		{ No visible objects <18 mag
345-1	2	17 July	01 56	-10		{ Comet Tempel 2 IR tail
363-1	2	26 July	14 02	-10		{ Possible Apollo asteroid observed at Palomar—lost
365-1	3	27 July	10 55	-67	17	Comet IRAS 1983o Recovered from
438-1	2	1 September	12 34	-51	17	ephemeris based on IRAS observations
418-1	2	23 August	03 32	+2	16	1983 QF main belt asteroid
460-1	2	12 September	03 54	+2		
424-1	2	25 August	03 28	+31		No object <18 mag
436-1	2	31 August	02 44	-10		1983 QG main belt asteroid
478-1	1	21 September	16 42	-24		Close to new comet Kowal-Vavrova
516-1	7	11 October	17 30	+59	15.5	No object seen
						1983 TB. Apollo asteroid/inactive comet. Parent body for Geminid meteor stream. Smallest known perihelion distance
559-1	2	1 November	22 10	-58	17	1983 VA. Apollo asteroid
577-1	2	10 November	21 33	-17	15	Comet Hartley-IRAS 1983v

Candidate moving objects were labelled according to the Spacecraft Operating Program (SOP) during which they were detected together with a sequential number within that SOP. Thus IRAS 180-1 was the first moving object alert originating from detections in SOP 180.

orbit under certain circumstances (that is the first detection is on the trailing edge of the array; the second detection is at the leading edge of the array). For the case with retrograde motion the limit is 15 arc min, but it will normally be less.

To minimize chance pairing of sources and to restrict motions to a limit which allowed a reasonable chance of recovery using Schmidt telescopes, the FMO detection software inter-compared all non-hours confirming sources within a search box of specified size. This search box was set throughout the mission to compare all sources within 25 arc min, both in right ascension and declination. Flux comparison tests were applied to pairs of sources separated by one or two orbits which satisfied these motion criteria. To allow for observational errors and possible rotational light-curve variations, flux differences were accepted up to a factor of 6.5 (that is, two magnitudes). All pairs of sources which passed the above tests were output by the software for individual identification. This checking eliminated known asteroids and comets (by comparison with daily ephemerides) and source confusion due to extended sources, such as dark nebulae (by examination of sky survey plates). Single detections of fixed sources which had 'leaked' into the software (for example, those awaiting confirmation on subsequent scans and which had been paired by chance) were also identified at this stage. Candidate moving objects which remained unidentified were communicated, together with an estimated visual magnitude based on the IR fluxes, to cooperating observatories with wide-field cameras for optical confirmation.

The program was first run using IRAS data on 11 February 1983, and, by the end of the month, known main-belt asteroids were being identified. Altogether, 37 alerts were sent to cooperating observatories, of which 17 were not searched for due to unfavourable position, bright moon, or bad weather. The alerts for which observations were attempted are summarized in Table 1. Further details of some strong unobserved or unconfirmed candidates are given in Table 2 for the information of observers who may have exposed plates near to these objects.

IRAS comets

Six comets were discovered by IRAS: the orbital elements derived from IRAS and ground-based measurements are given in Table 3. In addition, five known comets were identified from

published ephemerides; data for these are also given in Table 3. The new comets cover a range of inclinations, perihelion distances, and so on, and include both long- and short-period objects. There is a notable tendency for detection to occur at southerly latitudes, but this is more likely to be an effect of the IRAS survey strategy than an indication of the true distribution of cometary orbits. Although it will be necessary to carry out a detailed statistical study to be sure, it is not apparent on an initial examination that comets detected by IRAS have significantly different orbital characteristics from comets detected by observations in the visible.

In terms of individual characteristics, the first comet detected by IRAS (IRAS-Araki-Alcock) was notable because it passed close to the Earth shortly after its discovery, and so was studied in detail both by IRAS and by ground-based observers⁵⁻¹¹. Dynamically, comets 1983j and 1983v are more interesting. These are short-period comets with inclinations to the ecliptic >45°. Only one of the previously known short-period comets passing perihelion between 1982 and the end of the century (Comet Tuttle) has an inclination of this amount¹². In fact, Comet 1983v is the only short-period comet with an orbit nearly perpendicular to the ecliptic: it also has the shortest known period for a comet with a retrograde orbit. The question raised by these comets concerns their origin. Assuming that short-period comets are brought about by planetary capture, this can only have occurred when the comets were near the ecliptic plane. For Comet 1983j this is reasonable, because its aphelion distance is very close to the orbit of Saturn.

Comet 1983o was detected by IRAS on 27 July, but was not then recovered by ground-based telescopes. A subsequent IRAS detection on 1 September was recognized, from its IR fluxes, as being a second identification of the same object. An ephemeris based on the two positional measurements was then used to recover the comet from the ground¹³. This seems to be the first occasion on which a comet has been discovered and an ephemeris allowing recovery calculated purely on the basis of satellite observations.

The visual magnitude limit for a comet to be detected by IRAS is estimated to be ≈17. This is based particularly on observations of 1983f and 1983o, which were found to be close to the FMO detection threshold. However, the magnitude limit

Table 2 Strong unobserved or unconfirmed candidates

IRAS candidate	Julian date 244+	RA	Dec	Magnitude	Probable nature
201-1*	5460.11366	08h 40.12min	05° 28.30'	15	Asteroid
	5461.18812	08 40.96	05 26.10		
242-1*	5481.09294	09 46.75	-12 54.90	17	Comet
	5481.52749	09 46.88	-12 54.20		
272-1*	5496.11716	12 03.01	15 00.10	16	Asteroid
	5496.54680	12 03.34	14 55.00		
313-1*	5516.94787	12 21.97	10 48.80	17	Asteroid
	5517.01959	12 22.11	10 47.60		
305-1	5512.79523	13 00.91	15 51.20	17	Asteroid
	5512.86679	13 00.90	15 50.50		
354-1	5537.31041	01 42.46	-10 13.50	18	Apollo asteroid
	5537.38196	01 42.49	-10 12.90		
363-1	5541.93416	14 02.64	-10 22.00	18	Apollo asteroid
	5542.00585	14 02.30	-10 30.70		
429-1	5573.99831	14 12.92	-09 38.00	17	Apollo asteroid
	5574.06992	14 13.20	-09 40.50		
	5574.85718	14 17.48	-10 01.90		
	5574.92879	14 17.76	-10 03.90		
487-1	5603.76624	17 04.34	00 15.40	16	Apollo asteroid
	5603.83779	17 05.42	00 25.60		
	5603.90933	17 06.66	00 35.80		
	5603.98088	17 07.64	00 45.90		
	5603.05242	17 08.80	00 55.90		
576-1	5648.45677	09 29.15	08 12.20	16	Asteroid
	5648.52831	09 29.12	08 11.20		
588-1	5654.46718	10 07.74	07 09.00	17	Apollo asteroid
	5654.53878	10 09.23	07 18.20		

* Discovered during post-mission processing and not communicated to observatories.

Table 3 Orbits for IRAS comet discoveries

Comet	Perihelion time	Perihelion distance (AU)	Eccentricity	Period (yr)	Argument of perihelion (deg)	Longitude of ascending node (deg)	Inclination (deg)	Epoch	Ref.
1983d	21.2529 May 1983	0.991341	0.990115	1004	192.8439	48.4053	73.2495	26 May 1983	Marsden (1983) MPC 8272
1983f	19.0304 January 1983	1.416483	1.0	227.0690	118.9254	152.1948	152.1948		Marsden (1983) MPC 8052
1983j	23.8021 August 1983	1.696830	0.695573	13.2	356.8916	357.1602	46.1799	14 August 1983	Marsden (1983) MPC 8386
1983k	2.7224 May 1983	2.418226	1.0	265.5959	171.1037	138.8392	138.8392		Marsden (1983) IAU 3925
1983o	28.0267 November 1983	2.254646	1.0	333.9918	200.5600	120.7447	120.7447		Marsden (1983) MPC 8272
1983v	8.5225 January 1984	1.284336	0.836976	22.1	46.9272	0.7738	95.7675		Marsden (1983) MPC 8387
Known comets independently recovered by IRAS									
Pons-Winnecke	7.5035 April 1983	1.253991	0.634714	6.36	172.3194	92.7481	22.3081	15 April 1983	
Tempel-2	1.5355 June 1983	1.381404	0.544893	5.29	190.9220	119.1579	12.4375	25 May 1983	
Tempel-1	9.7951 July 1983	1.491120	0.520897	5.49	179.0424	68.3278	10.5553	4 July 1983	
Kopff	10.3389 August 1983	1.576273	0.544543	6.44	162.8143	120.3074	4.7245	13 August 1983	
Cernis	20.93178 July 1983	3.317031	1.0		186.15198	208.87845	134.69904		Marsden (1983) MPC 8139

Several known comets reaching perihelion in 1983, which may have been detectable by FMO software, were not recovered. These are: Comet Gunn 1969 II; Comet Johnson 1949 II; Comet Taylor 1961 I; Comet Schwassman-Wachmann (1) 1925 II; Comet Bowell-Skiff 1983c*; Comet Shoemaker 1983p; Comet Russell (3) 1983i; Comet Sugano-Saigusa-Fujikawa 1983e. The non-detections are attributable either to motions below the FMO detection threshold, or to the faintness of the object. The exception is Comet 1983e, which was both fast moving and very extended. In this instance, the comet may not have been scanned by IRAS, or have been recognised as a point source.

* Detected as a moving hours-confirmed source at SDAS.

obviously depends on the gas-to-dust ratio, as the IR emission comes from the heated dust. A dusty comet could be detected at somewhat fainter visible magnitudes, whereas a mainly gaseous comet would be visually brighter than the proposed limit. As we have noted, detection of comets is limited not only by brightness, but by motion. Thus Comet 1983t was probably not detected by the FMO software. There has been some confusion on this point as an ambiguous IRAS detection led to a ground-based observation of the comet. However, this was probably a coincidence: not only was the comet of visual magnitude 18 at this time, but its motion (41 arc s h^{-1}) was then below the detection threshold of the FMO software.

There may well be other comets brighter than $M_v = 17$ with motions below 1 arc min h^{-1} in the IRAS database. These were not processed by the FMO software because they passed the hours-confirmation test. It is clearly important to determine whether they will be identifiable during the analysis of slow-moving objects at SDAS. Main-belt asteroids processed by the FMO software exhibited a range of IR flux ratios, due to their range of temperatures and to errors within the IRAS detectors.

These were wide enough to encompass all the comet detections except IRAS-Araki-Alcock (whose proximity to Earth resulted in a significantly higher effective temperature). It might therefore appear that moving objects, which are recognized as essentially point sources by IRAS, cannot be separated into comets and asteroids: this has to be done by ground-based observation. In fact, the two classes of objects actually can be distinguished by examination of the unprocessed detector output. As comets are extended sources, the profile of a detection in the in-scan direction is wider than that of a point source. In the cross-scan direction, detectors adjacent to those along the nominal source track may show evidence of a signal, revealing an angular size greater than that of a single detector element. This test was successfully applied to Comet 1983f, which was the faintest of the IRAS comets.

Note that almost all moving, three-band sources detected by the FMO software were identified as known main-belt asteroids. Thus, any such source which cannot be so identified in the IRAS output has a good chance of being a comet. It should be checked for spatial extension by reference to the IRAS unprocessed data.

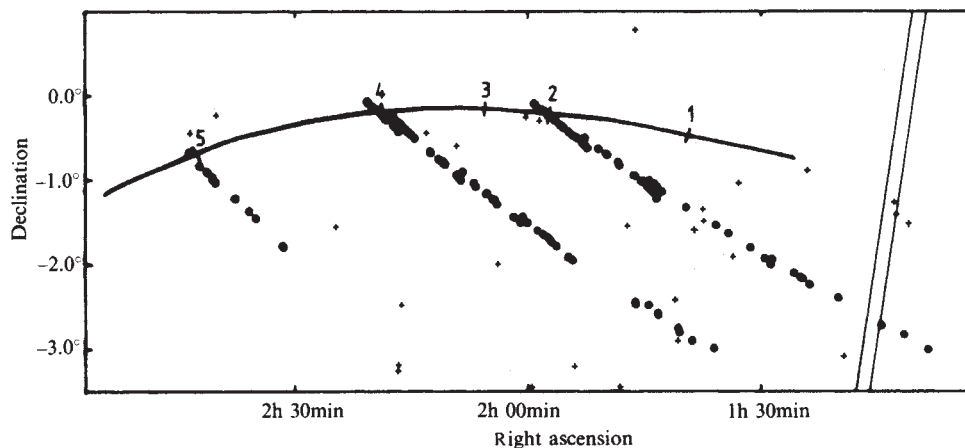


Fig. 1 Positions of non-hours-confirmed sources detected 10–17 July, 21–27 July and 8–9 August. ●, Detections of the tail of Comet Tempel-2; +, other sources. Positions of the comet on (1) 10 July, (2) 17 July, (3) 21 July, (4) 27 July, (5) 9 August, are marked along its track. A single IRAS scan track is also shown at the right-hand side of the diagram.

This should also be done for apparently spurious sources detected during IRAS additional observations: we urge IRAS AO observers to bear this possibility in mind when examining their data.

IR tail of comet Tempel-2

During the period 12–18 July, the FMO software detected about 50 faint, seconds-confirmed sources, having a signal-to-noise ratio of ~ 5 in the IRAS 25 μm band. These sources seemed to be related to the periodic comet Tempel-2. Figure 1 shows a sample of the detections together with the position of the Tempel-2 nucleus at the times corresponding to their detection. Note that the IRAS scan crosses the tail almost at right angles. With the $\sim 1^\circ \text{ day}^{-1}$ progression of the IRAS scan, several days were required to build up a full picture of these features (see Fig. 1). That they represented sections through a continuous tail was verified during the repeat survey scans for weeks confirmation (22–27 July). These showed features in a similar sequence relative to the comet (which had, of course, moved relative to the stars during the intervening period). An examination of the raw data subsequently indicated that the tail was recorded by all band II detectors across the focal plane. Only the most sensitive of these registered a signal-to-noise ratio > 5 , the PAF processing threshold.

The width of the Tempel-2 tail was found to be ~ 4 arc min, although its angular length was $\sim 10^\circ$. This clearly represents an unusually high aspect ratio. The position of the tail was passed to ground observers, but no optical detection was reported (although a 4 arc min tail was reported by a visual observer¹⁴).

No similar feature was detected for any of the other comets observed by IRAS. Analysis is under way to determine whether the detection was an effect of optical depth due to the proximity of the Earth to the comet's orbital plane, or whether it was due to an outburst of material in the days preceding the IRAS detection.

Earth-crossing asteroids

It was expected that Earth-approaching asteroids (Apollo asteroids) would dominate the objects discovered by the FMO software. However, as indicated in Table 4, only two IRAS FMO

candidates have been optically confirmed as new Apollo objects. In addition, the software independently discovered Apollo asteroid 2201 Oljato, whilst another strong candidate (IRAS 487-1) was not optically recovered.

The small number of detections compared with pre-launch predictions can be attributed to a variety of factors. In particular, the predictions were based on an estimate of the sensitivity in the 12- μm band which proved overoptimistic by approximately a factor of two. In addition, the diffuse 25- μm background, especially near the ecliptic plane, made identification based only on 25- μm detections very uncertain. It should be added that, towards the end of the mission, the scan strategy was changed from coverage of lunes (areas bounded by lines of longitude 60° apart) to scans over the ecliptic poles, resulting in greater sky coverage at high ecliptic latitudes.

Detection of Earth-crossing asteroids also depends critically on their motion. Thus, Apollo asteroid 1620 Geographos was seen only once, and asteroid 1983LC escaped detection totally, because their motions were of the same order as the width of the IRAS scan, but in the opposite direction to that in which the orbital overlap advanced. This allowed them to cross the scanned region between successive IRAS orbits. Conversely, objects whose motions were approximately coincident in direction with the scan advance were detected on several successive orbits, for example 2201 Oljato (six detections), IRAS 487-1 (five detections), 1983TB (seven detections).

As shown in Table 4, the orbital elements of 1983TB are similar to the mean orbital elements of the Geminid meteors^{15,16}. The implication is that 1983TB could be the progenitor of the Geminids¹⁷, and may therefore represent a decayed, or inactive, cometary nucleus. No evidence of cometary activity was reported by ground observers, and the source appeared point-like in the IRAS data. The Geminids differ from most meteor showers in the apparently higher mean density of the particles. Hence, we may be seeing here an unusual type of meteor source.

Conclusions

The IRAS mission has provided an all-sky IR survey with a detailed pointing history. The analysis now in progress will allow an estimate to be made of the detectable population of comets

Table 4 Orbital elements of asteroids discovered by IRAS

Asteroid	Absolute magnitude	Epoch	Mean anomaly	Argument of perihelion (deg)	Longitude of ascending node (deg)	Inclination (deg)	Eccentricity	Mean distance (AU)	Ref.
1983 QF	12.5	3 September 83	323.52	277.46	167.34	22.66	0.2164	2.6909	Marsden (1983) MPC* 8207
1983 QG	14.5	27 October 84	98.80	279.55	80.24	14.30	0.3463	2.6430	Marsden (1984) MPC 8467
1983 TB	16.0	27 October 84	290.30	321.69	265.03	22.04	0.8903	1.2715	Marsden (1983) MPC 8394
Geminids	—	—	—	324.8	260.6	23.6	0.896	1.35	I. P. Williams (personal communication)
1983 VA	17.5	2 November 83	349.26	11.74	76.94	16.20	0.6888	2.5821	Marsden (1983) MPC 8395

* Minor planet circulars, Smithsonian Astrophysical Observatory.

and Apollo asteroids. It is, however, possible to draw the following general conclusions from the results already obtained with the FMO programme.

- (1) IRAS could detect comets having visual magnitudes <17 , provided their motions exceeded $\sim 1 \text{ arc min h}^{-1}$.
- (2) The positional accuracy of IRAS detections depended on the number of bands in which an object was observed. The accuracy proved adequate for approximate ephemeris calculation, though unsuitable for accurate orbit determination.
- (3) There is no evidence for a significant population of undiscovered main belt-asteroids at high ecliptic latitudes, at least amongst those having visual magnitudes brighter than 16 and

motions $\geq 1 \text{ arc min h}^{-1}$.

(4) Asteroids and comets are both identified by PAF as point sources. The two types can, however, be distinguished by referring back to the raw data where these are available.

The success of this project would not have been possible without the support and cooperation of the IRAS team at RAL and the help of optical astronomers around the world who reacted promptly to IRAS alerts. We thank especially B. G. Marsden and D. Green of the Central Bureau for Astronomical Telegrams for their invaluable assistance. IRAS is a joint project of US NASA, the Netherlands NIVR and UK SERC. J.K.D. and S.F.G. thank SERC for financial support during this project.

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ARTICLES

Interaction of small and extended defects in nonstoichiometric oxides

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Analyses of the extrinsic and/or intrinsic nature of extended defects in nonstoichiometric titanium and tungsten oxides are made by drawing Burgers' circuits directly onto high-resolution electron micrographs. The essentially interstitial and/or vacancy nature of the small defects responsible for the phases TiO_{2-x} and WO_{3-x} , which exist above the extended defect precipitation temperature, may then be implied. Images suggesting a partial wall of anion vacancy defects have also been obtained.

CONSIDERABLE uncertainty and controversy surrounds the nature of the small defects responsible for the nonstoichiometric oxides TiO_{2-x} and WO_{3-x} (refs 1, 2). Thermodynamic studies^{3,4} and physical property measurements (such as electrical conductivity^{5,6}) are interpreted traditionally in terms of point defect structures; namely, singly- or doubly-ionized oxygen vacancies or cation interstitials. Mechanisms of aggregation or interaction of small defects to precipitate as extended defects have been derived for both oxide systems^{7,8}, but it has always seemed possible to conceive of alternative or competing mechanisms; one based essentially on charge-compensated oxygen vacancies^{9,10}, and one based on electrostatically neutral linear cationic interstitial defects^{7,8}. We show here that whether crystallographic shear planes (CSP) grow by aggregation of interstitial and/or vacancy defects can be established by determining the extrinsic or intrinsic nature of the partial dislocation bounding the CSP, as revealed by drawing Burgers' circuits directly onto high resolution micrographs. This represents one approach to determining the nature of the small defects.

High resolution electron microscope (HREM) studies of TiO_{2-x} and WO_{3-x} have recently established structural features that were overlooked by earlier lower resolution studies. In the case of slightly-reduced rutile, TiO_{2-x} ($0 \leq x \leq 0.0035$), these include the existence of {100}-platelet defects¹¹ and the precipitation of pairs of CSP in slowly-cooled samples¹². In WO_{3-x} , at least three types of extended defects have been observed:

CSP^{13,14}, pentagonal bipyramidal columns (PC)^{10,15} and hexagonal tunnels (HTB)¹⁶. These phenomena were all consistent with new linear interstitial cationic defect models, derived to explain the structure of the small defects in TiO_{2-x} (refs 7, 9).

Experiments

High-resolution images were obtained with the Cambridge University 600-kV HREM¹⁷. As this microscope has an interpretable resolution limit of better than 2 Å, cation arrangements along CSP can be read directly for appropriately focused images of sufficiently thin crystals¹⁸. Figures 1a, b show examples for a [111] specimen of $(\text{Ti}, \text{Cr})\text{O}_{1.92}$ and a [100]_{pc} specimen of $(\text{W}, \text{Nb})\text{O}_{2.933}$ respectively. (Further details of specimen preparations and imaging conditions are given in ref 19. Indices without subscripts refer to rutile whereas those subscripted pc refer to pseudocubic axes of WO_{3-x} .) Disorder within the CSP is readily visible in TiO_{2-x} (Fig. 1a; see ref. 12 for structural interpretations) and Fig. 1b shows evidence for split occupancy of one double-square tunnel (labelled S), corresponding to steps in CSP structure for WO_{3-x} (work in preparation).

Many CSP terminations have been imaged for specimens of $\text{TiO}_{1.9966}$ (ref. 19) and $(\text{W}, \text{Nb})\text{O}_{2.933}$. In each case differences in detail occurred so that it became clear that there was no unique partial dislocation structure. Thus CSP in rutile were often 'decorated' by {100} platelet defects^{11,19} (Fig. 2), whereas CSP in tungsten oxides often contained pentagonal columnar

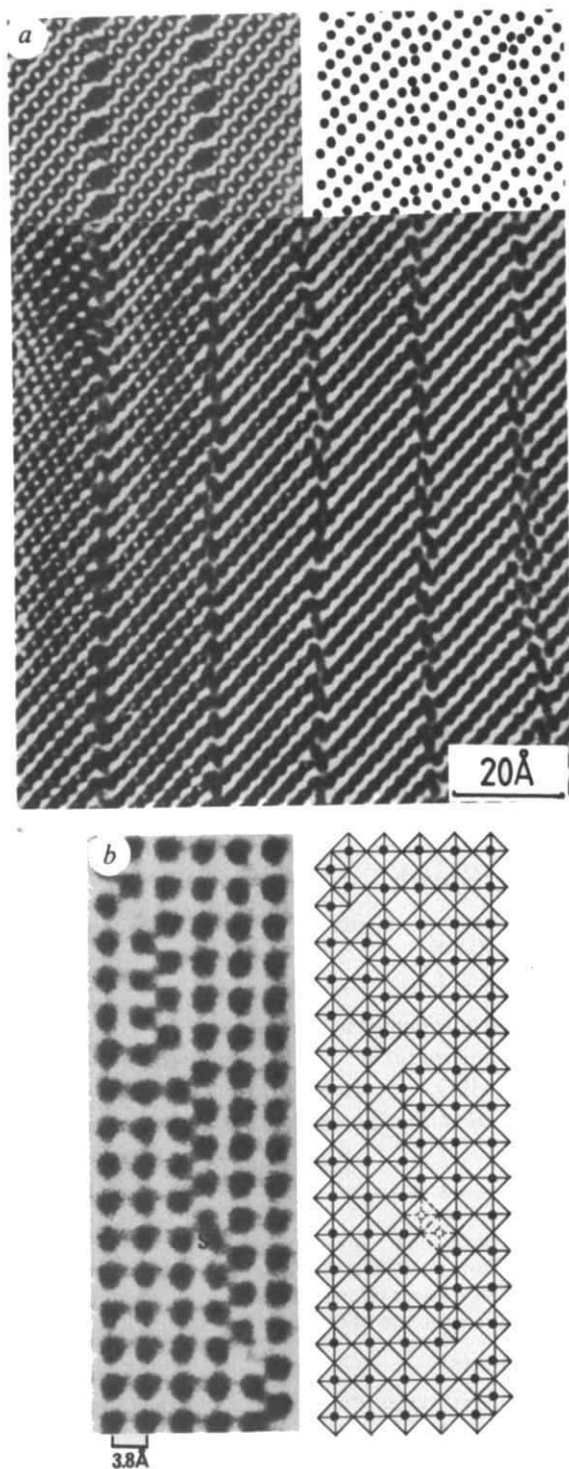


Fig. 1 *a*, 500-kV image of $(\text{Ti, Cr})\text{O}_{1.92}$, showing slightly disordered crystallographic shear planes (CSP). Inset shows the structural model for ordered (374) CSP and the computer simulation. Note the sequence of occupied cation sites, which may be read off the image provided the disorder is not excessive. *b*, 500-kV image of $(\text{W, Nb})\text{O}_{2.933}$ showing a segment of $(103)_{\text{pc}}$ CSP. The inset gives the octahedral representation, showing that tungsten (or Nb) cation positions are located readily by inspection of the image.

elements within the termination (Fig. 3). These two examples have been chosen for analysis here because of their relative simplicity; further variations, with structural interpretations, are given in ref. 19.

It was surprising that, for both systems, extended defects occurred which exhibited approximately zero lattice translation, corresponding to pairs of CSP in rutile (see ref. 19) and pairs of occupied pentagonal tunnels for tungsten oxide (Fig. 4).

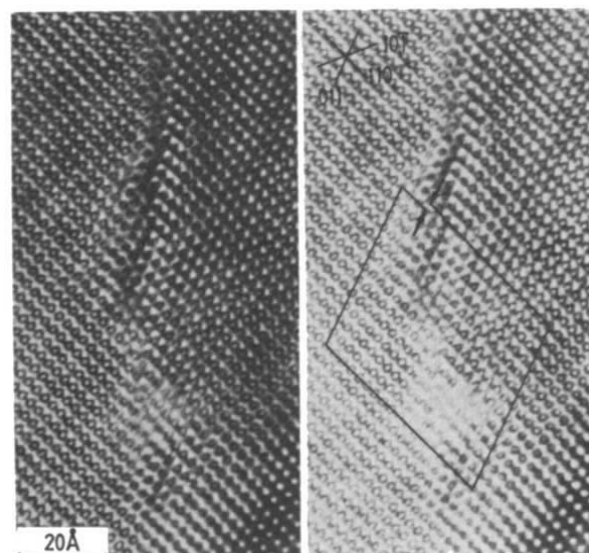


Fig. 2 500-kV image of $\text{TiO}_{1.9966}$ (reduced at 1,323 K and cooled slowly over 1 week in a dynamic vacuum of 10^{-6} torr)¹¹ showing termination of single (132) CSP. The inset shows Burgers' circuit analysis, with closure failure $\sim [0\frac{1}{2}\frac{1}{2}]$, establishing extrinsic nature for this CSP.

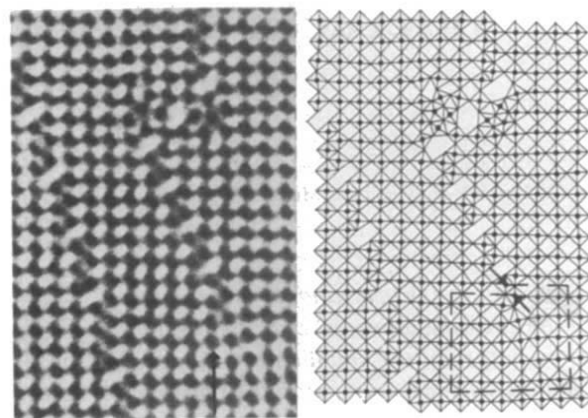


Fig. 3 500-kV image showing termination of $(103)_{\text{pc}}$ CSP within WO_3 . The location of the terminating half-plane (sight along arrow) establishes the intrinsic nature of this CSP. The inset shows Burgers' circuit analysis, with closure failure $[\frac{1}{2}\frac{1}{2}0]_{\text{pc}}$ (see Fig. 2).

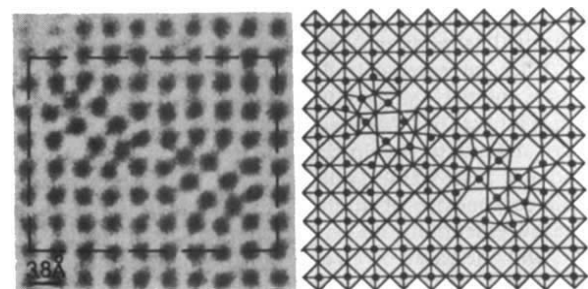


Fig. 4 500-kV image of $(\text{W, Nb})\text{O}_{2.933}$ showing two pairs of pentagonal bipyramidal columnar defects; occupied tunnel sites are located by comparison with the structural drawing. Burgers' circuit shows zero lattice displacement.

Burgers' circuit analyses

The geometrical operations for single CSP in TiO_{2-x} and WO_{3-x} may be written $(132)[0\frac{1}{2}\frac{1}{2}]$ or $(100)_{\text{pc}}[\frac{1}{2}\frac{1}{2}0]_{\text{pc}}$ for extrinsic, and $(132)[0\frac{1}{2}\frac{1}{2}]$ or $(100)_{\text{pc}}[\frac{1}{2}\frac{1}{2}0]_{\text{pc}}$ for intrinsic defects respectively. A convention is adopted that if the CSP has indices (hkl) and displacement $[uvw]$ then the dot product $(hkl) \cdot [uvw]$ is >0 for extrinsic and <0 for intrinsic CSP. In the case of rutile, diffrac-

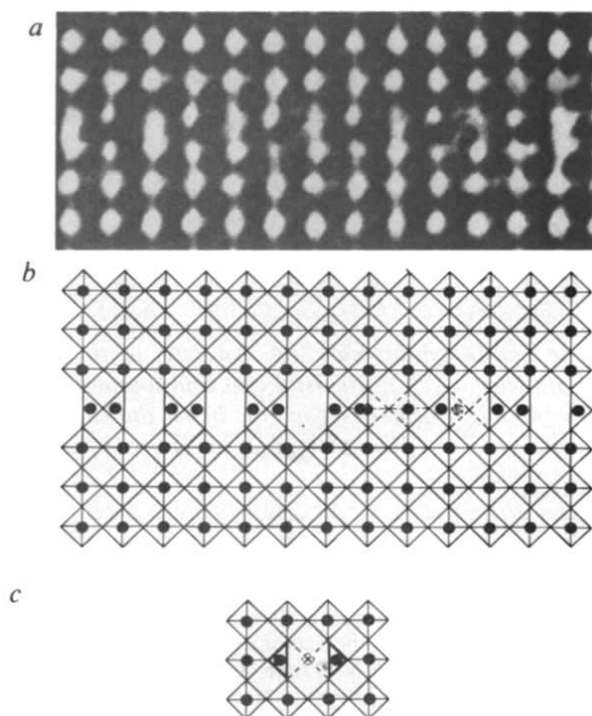


Fig. 5 500-kV image of $(\text{W, Nb})\text{O}_{2.933}$ showing novel defect contrast in WO_3 matrix between CSP (a), which may be interpreted as a partial wall of oxygen vacancies aligned onto $(100)_{\text{pc}}$, premonitory to collapse and formation of a CSP (b). Some apparently split-tungsten atom images indicate partial emptying of oxygen sites along projection axis (indicated in b). c, A structural model for a single oxygen vacancy defect showing relaxation of two adjacent M^{5+} cations into square pyramidal coordination. The relaxation is estimated to be 20% of 3.8 Å (=0.8 Å).

tion contrast experiments gave $[0, \overline{0.45}, 0.45]$, assuming intrinsic CSP²⁰, and it is the difference between this and the alternative extrinsic vector $[0, 0.55, \overline{0.55}]$ that may be distinguished by direct imaging of the termination. The principles of the Burgers' circuit analysis may be seen by inspection of the drawings given with Figs 2, 3. Thus, starting the Burgers' circuit in a region of perfect crystal, some distance from the defect, and returning to this vicinity, having crossed the single CSP (Figs 2, 3) or circumnavigated the CSP pairs¹⁹ or pentagonal columns (Fig. 4), one finds that a slab of crystal having thickness $(hkl) \cdot [uvw]$ has been added for rutile (Fig. 2) but eliminated for tungsten oxide (Fig. 3) for single CSP, but there is no lattice translation for the defect pairs.

Note that the component of lattice translation parallel to the projection axis is half a lattice vector, for both CSP structures, and is zero for pentagonal columns. Otherwise it would be necessary to image each defect in at least one other projection to measure a third component of the Burgers' vector.

Discussion

It is remarkable that TiO_{2-x} and WO_{3-x} yield opposite characteristics for the terminations of single CSP, implying that the nature of the small defects should be essentially cation interstitial for TiO_{2-x} and essentially oxygen vacancy for WO_{3-x} . For

TiO_{2-x} the linear small defect cationic interstitial model⁷ also explains the structure and growth of CSP pairs^{7,19}. Precipitation of pairs of occupied pentagonal columns in WO_{3-x} is not consistent with oxygen vacancy defects⁸, but is explained readily using a linear cationic defect model²¹. Thus the Burgers' circuit shown in Fig. 4 contain two columns having occupancy $-\text{O}-\text{M}-\text{O}-\text{M} \dots$, in addition to the host WO_3 structure. On the other hand, the presence of single intrinsic CSP in WO_{3-x} implies oxygen vacancy defects.

Strong evidence for the existence of oxygen defects in WO_{3-x} was found inadvertently (Fig. 5a). This novel type of defect contrast was located close to the well-known CSP and PC features. Direct image interpretation on the basis of a structural drawing alone, as carried out above (see Figs 3, 4), seems justified although computer simulations will be used to provide further confirmation. Thus the occurrence of double-tunnel contrasts, aligned onto $(100)_{\text{pc}}$, with displacements of adjacent black blobs (usually tungsten ion positions), may be interpreted (Fig. 5b) as representing a partial wall of oxygen vacancies, accompanied by relaxation of adjacent cations (ideally Nb^{5+} ions) into pairs of adjacent square pyramidal five-coordinated sites. This gives rise to a charge-compensated oxygen vacancy model (Fig. 5c). (In practice, at elevated temperatures Nb^{5+} cations may be expected to become detached from the vacancies, being replaced by W^{6+} .) This last observation, together with further results (in preparation), imply that single CSP grow within WO_{3-x} by aggregation of oxygen vacancy defects, either feeding stepwise into existing CSP to be first forming partial walls of oxygen vacancies, premonitory to collapse and production of intrinsic CSP, bounded by a partial dislocation loop (see refs 21, 22).

It would be foolish to claim that in either oxide system, there is a unique CSP, or other extended defect, precipitation mechanism, or that there is only one small defect type in either non-stoichiometric phase. Certainly, in the case of rutile, there appears to be three regimes, where the small defect appears to be predominantly interstitial, predominantly vacancy, or impurity controlled, respectively^{5,6,9}. To date HREM analyses have been restricted to specimens cooled from within the interstitial regime. For WO_{3-x} the present observations suggest that oxygen vacancy defects occur, but that interstitial defects may be required for precipitation of PC. It may be that the former occur predominantly on irradiation in the electron beam (see ref. 23) and that specimens cooled slowly across the phase boundary will show evidence for CSP-pair precipitation⁸.

Conclusion

The extrinsic or intrinsic nature of extended defects may be determined by drawing Burgers' circuits onto HREM images. Such analyses may also yield, by implication, the nature of small defects existing within nonstoichiometric phases just before precipitation. It seems unlikely that unique and reproducible CSP partial dislocation structures occur in either TiO_{2-x} or WO_{3-x} , the structure of individual defects being dependent on the relative concentrations of different small defect types existing locally at the time of precipitation. Further attempts to obtain specimens and images from different regions spanning the non-stoichiometric phases are planned, but this will require even greater control of specimen preparation conditions than used so far, especially for WO_{3-x} where the phase limits are poorly defined⁴.

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Somatic recombination in a murine T-cell receptor gene

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A putative T-cell receptor gene was isolated from the DNA of the helper hybridoma, 2B4. Analysis of the sequence components of this rearranged gene indicates the presence of separate variable (V), diversity (D) and joining (J) region elements analogous to those of heavy-chain immunoglobulins. These findings further support the belief that the products of this locus are involved in antigen recognition by T cells. There is no evidence of somatic mutation between the putative germ-line and the expressed variable-region gene.

T LYMPHOCYTES have a variety of important roles in the immune system of higher organisms, including the direct killing of virally infected cells (cytotoxic T cells) and either amplification (helper T cells) or inhibition (suppressor T cells) of the response to antigen. In order for T cells to perform these functions, they require a receptor for antigen which allows them to distinguish 'self' from 'non-self'. While the T-cell receptor appears similar to that of B cells (antibody) in that it is clonally distributed and has a wide range of specificities, it is functionally distinct in that it can only recognize antigen in association with a product of the major histocompatibility complex (MHC): the H-2 region in the mouse and the HLA region in man. Particular subsets of T cells use different MHC molecules; cytotoxic T cells require class I molecules (H-2K, D, L in the mouse) and helper T cells require class II molecules (H-2A and E in the mouse). Any structural model of the T-cell receptor(s) must explain how these types of T cell can recognize antigen only in conjunction with self-MHC, yet respond strongly to MHC determinants from other individuals (alloreactivity) without previous exposure to these antigens, and how the ability to 'learn' self-MHC occurs in the thymus, also independent of antigen. There are two major schools of thought which attempt to explain these phenomena: the 'one receptor' hypotheses¹ hold that T cells recognize a complex of antigen plus MHC ('altered self') while the 'two receptor' models² propose separate receptors for antigen and MHC ('dual recognition'). Recent evidence from cloned T-cell lines and hybridomas strongly favours the view that the receptor(s) cannot recognize antigen or MHC independently³⁻⁵, although there are contradictory data from other sources^{6,7}.

Recently, Hedrick *et al.*⁸ isolated a cDNA clone (TM86) which is T cell-specific, localized on membrane-bound polysomes and systematically rearranged in a variety of T-cell lines and hybridomas. Sequence analysis of this and related cDNA clones (86T1, 86T3 and 86T5) indicates significant similarity to immunoglobulin variable and constant regions and an independently assorting 16-amino acid element homologous to both heavy- and light-chain J regions⁹. Antisera raised against synthetic peptide fragments of this molecule significantly inhibit the *in vitro* response of certain T-helper (T_H) hybridomas (J. Rothbard *et al.*, in preparation). Thus, it seems probable that these cDNAs encode one chain of the murine T-cell antigen receptor. Other workers¹⁰ recently isolated a cDNA clone from a human T-cell lymphoma line which appears to encode a closely related molecule. To investigate the structure of the genes encoding these molecules and the rearrangements that occur at the genomic level during T-cell differentiation, we examined the pigeon cytochrome *c*-specific, H-2E-restricted T_H hybridoma, 2B4 (ref. 11). A monoclonal antibody specific to this particular line has been isolated and shown to precipitate a 43,000 (43K)-molecular weight disulphide-linked heterodimer¹¹ characteristic of the murine receptor for antigen^{12,13}.

Sequence of a 2B4 cDNA clone

A library of cDNA clones from 2B4 mRNA was constructed as described elsewhere¹⁴ in the vector λ gt-10 (ref. 15). Phage were screened with 86T5, a cDNA clone largely containing constant-region sequences of the putative receptor molecule⁹. Approximately 0.02% of the clones were positive, of which two distinct types were evident: one of these derives from the fusion partner BW5147 and will be described elsewhere—the other type appears to be specific to the 2B4 hybridoma and the structure and sequence of a representative clone, p2B4.71, is shown in Fig. 1. The amino acid numbering system is that of the mature protein, assuming a signal peptide cleavage 19 amino acids from the initiation codon⁹ (by analogy to immunoglobulin). Unlike the previously described murine cDNAs⁹, p2B4.71 possesses a complete 3' end with a stop codon after amino acid 287 and a 3'-untranslated region of 174 nucleotides, ending with a poly(A) addition signal 17 nucleotides 5' to the poly(A) tail. The 5' end is not complete as shown, but we were subsequently able to deduce the remaining variable region—leader polypeptide sequence from the genomic clones (see below).

The amino-terminal portion of the constant region (amino acids 115–241) has only one nucleotide change (silent) at amino acid 201. However, after position 241, the nucleotide sequences diverge significantly (15%) (Fig. 1). Interestingly, the amino acid sequence is relatively unchanged (only four amino acid changes result from 22 nucleotide changes). Although these differences could have been due to divergence between strains of mice in a single gene (86T1 is from BALB/c and p2B4.71 is from B10.A), our recent data indicate that the constant regions of these cDNAs are derived from two different genes and that the regions of similarity and divergence are encoded in separate exons⁴².

Also interesting is the fact that the same J region used in TM86 (ref. 9) appears again in the 2B4.71 sequence but joined to a different variable region, further confirming the ability of these elements to assort independently of the variable regions. TM86 derives from a T_H hybridoma, 3.3T (ref. 4), which responds to a cytochrome *c* fragment different from that of 2B4, but using the same MHC backgrounds (B10.A (2R) for 3.3T and B10.A for 2B4). While the 5' regions do not appear to be similar, it may be significant that they share the same J region. In contrast to the TM86 fragment, the p2B4.71 variable region shows significant similarity to that of 86T1, which was obtained randomly from a thymocyte library⁹. Homologous residues are indicated by asterisks in Fig. 1. In all, 31/90 (34%) residues in the portion of variable region shown are identical; most of these are located in the same regions that are relatively invariant (framework residues) in both heavy- and light-chain immunoglobulins¹⁶. Although it will be necessary to sequence many more T-cell variable regions to be certain, this comparison indicates that the structure of the receptor binding site may be very similar to

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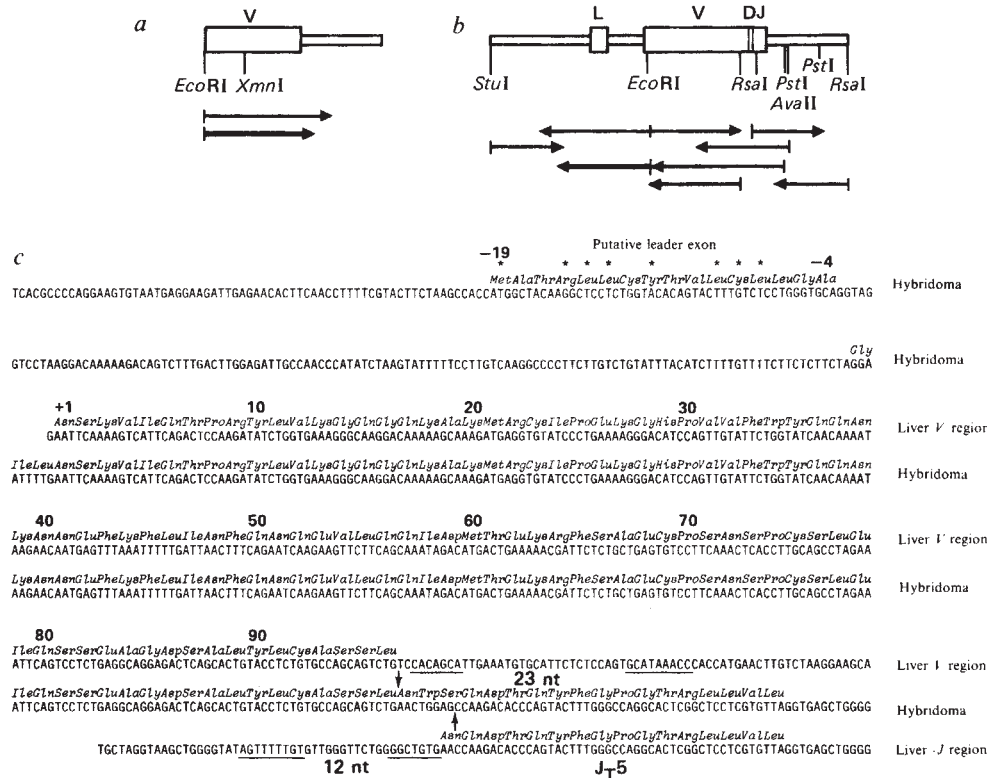


Fig. 3 Assembly of the 2B4 variable region. The sequencing strategy for the liver (a) and 2B4 (b) derived variable-region fragments. The J-region sequence is taken from the data presented in Fig. 4. Thin lines indicate 5'-end labelling with polynucleotide kinase and thick lines indicate 3'-end labelling with the large (Klenow) fragment of DNA polymerase I¹⁹. The sequences are shown in c, with the apparent leader exon (residues homologous to the 86T1 leader are marked with asterisks) and the variable, diversity and joining region components as indicated. Putative signal sequences for DNA rearrangement, separated by 23 and 12 nucleotides (nt) respectively, are underlined. Arrows indicate the apparent points of rearrangement.

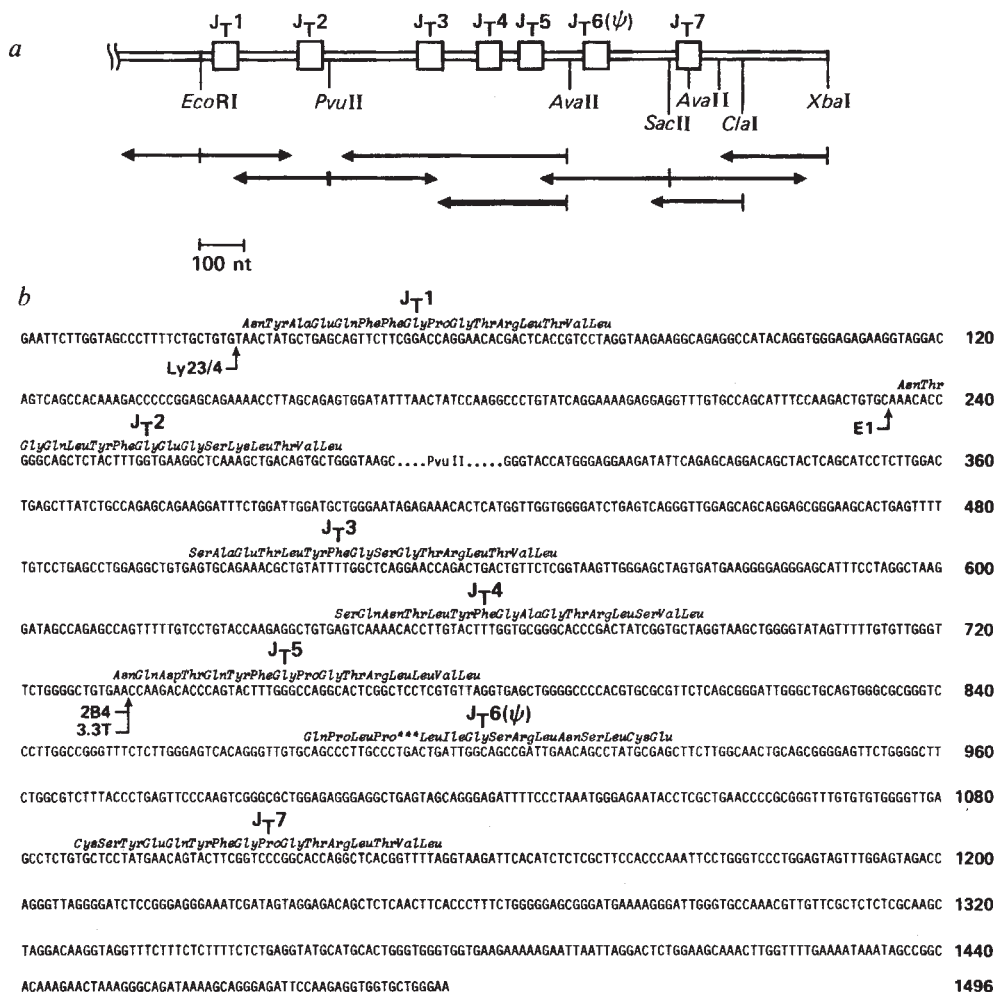


Fig. 4 J-region cluster. The fragment indicated from the genomic clone B.22 was sequenced by the strategy shown in a. Thin lines indicate 5'-end labelling and thick lines represent 3'-end labelling. Apparent J_T elements were identified based on amino acid and flanking sequence homologies to expressed J_T regions⁹. The stop codon in the J_T6 pseudogene is indicated by ***. The apparent points of rearrangement of p2B4.71(2B4) and of TM86⁹ (3.3T) as well as those of cDNA clones derived from E1 (ref. 40) and Ly 23/4 (ref. 41 and P. Patten *et al.*, unpublished results) are indicated by arrows.

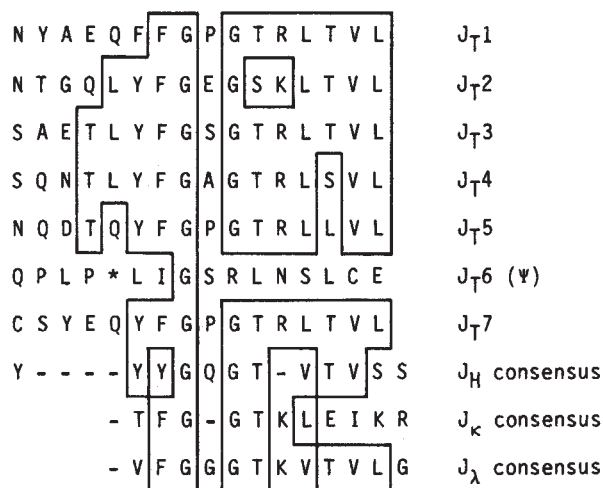


Fig. 5 Amino acid homologies between the seven *J*-region elements. Comparison of the coding sequence homologies between the *J* regions and the consensus sequences from the three different types of immunoglobulin¹⁶.

has to that of 86T1 (ref. 9) (8/15) residues at identical positions). This assignment is probably correct because of the virtual identity that these presumptive intron-exon boundaries have with immunoglobulins²⁰.

Figure 3c also shows that an 8-nucleotide sequence at the 3' portion of the rearranged variable region is not derived from either of the two liver DNA sequences. This sequence is at a position similar to the *D* or diversity region in immunoglobulin heavy chains and codes for a similar number of residues²¹⁻²⁵. Although we cannot exclude the possibility that this extra sequence is not encoded in the germ line²⁶, it does bear an intriguing resemblance to that of an immunoglobulin *D* element, Q52 (CAACTGGAC)²². As recent data show that this gene is not located on the same chromosome as the mouse heavy-chain locus⁴³, this homology might derive from some functional constraints on the *D* region common to both the T-cell receptor and immunoglobulins. The existence of a family of such elements for the T-cell molecule could greatly increase the number of possible variable regions by at least three mechanisms known from the example of immunoglobulin genes: combinatorial association of a third genetic element²¹, variability in the junction points at the DNA level^{21,23}, and the possibility of reading in different frames²⁷.

It is important to compare the unrearranged *V* region with the corresponding 294 nucleotides of the hybridoma gene. Within this region there is complete homology between the hybridoma and liver-derived gene sequences. This indicates that no somatic mutation has occurred between the putative germ-line and the active gene, and while this does not rule out a role for such mutation in other contexts, it does make some of the more extreme models of receptor adaptation to self-MHC and antigen less likely²⁸.

Also significant is the fact that by combining the complete, processed *V(DJ)* region sequence shown in Fig. 3c with the complete constant region of Fig. 1b, we can deduce a molecular weight of 32.1K. This is in very close agreement with the value of 31K obtained in tunicamycin studies of the unglycosylated murine receptor peptides²⁹.

Directly 3' to the *V* region of the germ-line DNA in Fig. 3c is the sequence CACAGCA-[23 nucleotides]-GCATAAACC which is similar to the putative signal sequences for gene segment fusion 3' to the immunoglobulin *V* regions^{30,31}. In immunoglobulins, the sequence is typically CACAGTG-[11 or 22 nucleotides]-ACAAAACC 3' to the *V*^{22,27}; the inverse of the putative *J*-region signal sequences (see below). Although the analysis of one *V* region is insufficient to establish exact sequence requirements, it seems significant that the last two nucleotides of the putative *V*-region heptamer (CA) and the first nucleotide of the nonamer (G) have never appeared in those positions of the relevant immunoglobulin sequences. These similarities and differences compared with immunoglobulin flanking sequences are discussed more extensively with the *J*-region data below.

J-region cluster

Sequence analysis of the liver DNA-derived clones in the region immediately surrounding the point of rearrangement (Fig. 1) indicates the presence of a cluster of seven *J* regions. Three of these *J* regions (J_T1, J_T2 and J_T5) have been found expressed in different cDNA clones (see Fig. 4 legend), and as many as six may be functional. J_T6 is clearly not functional because of the in-frame stop codon. Figure 4 shows the sequencing strategy of the 1.8-kb fragment containing the *J* regions. The J_T cluster begins ~3.6 kb 5' to the first constant-region domain⁴². The J_T elements are very closely spaced at 40–220 nucleotides apart, whereas immunoglobulin *J* regions are typically 300–500 nucleotides apart^{22,30,31}. The *J* region used by p2B4.71 and TM86 (ref. 9) is the fifth in the cluster (J_T5) and, as indicated in Fig. 4, joining occurs at the same position for each. Of particular interest are the putative signal sequences for gene segment fusion 5' to each *J* region. In immunoglobulins, these sequences, spaced either 10–12 or 21–23 nucleotides apart, are highly conserved between all functional *J* regions in a variety of species^{21,22,30,31}. The sequence is typically GGTTTTTGT-[11 or 22 nucleotides]-CACTGTG 5' to *J* or *D* elements and the inverse: CACAGTG-[11 or 22 nucleotides]-ACAAAACC 3' to *V*. The sequence 5' to three of the *J* regions in Fig. 4 is AGTTTTTGT-[12 nucleotides]-GGCTGTG. The other sequences diverge slightly from this consensus. Although these sequences are very similar to those of the immunoglobulin genes in all cases, the heptamer is not palindromic and the sequence 'GG' has not been found in any of the many immunoglobulin sequences in this position in either mouse, human or rat¹⁶. The region immediately 3' to the apparent functional *J* regions also contains conserved sequences, as indicated in Fig. 4, but these may have more to do with RNA splicing than recombination. We conclude that the gene segments examined here have sequences which are analogous to those used by immunoglobulins for variable-region assembly but that they differ slightly in the conserved sequence,

9	(10-14)	7	<i>J_T</i> coding regions	
ATTCTTGGT	AGCCCTTTTC	TGCTGTG	TAACTATGCTGAGCAGTTCTTCGGACCAAGAACACGACTCACCGTCTCTAG	GTAAGA
	(AT)			
GAGGTTTGT	GCCAGCTTCAA	GACTGTG	CAACACCGGGCAGCTCTACTTTGGTGAAGGCTCAAAGCTGACAGTGCTGG	GTAAGC
AGTTTTTGT	CCTGAGCCTGGA	GGCTGTG	AGTGCAGAAACGCTGATTTTGGCTCAGAACCAAGCTGACTGTTCTCG	GTAAGT
AGTTTTTGT	CCTGTACCAAGA	GGCTGTG	AGTCAAAACACCTTGACTTTGGTGGGACCCGACTACGGTGCTAG	GTAAGC
AGTTTTTGT	GTTGGGTTCTGG	GGCTGTG	AACCAAGACACCCAGTACTTTGGGCCAGGCACTCGGCTCCTCGTGTAG	GTAGAC
	(AG)			
CGGGTTTCT	CTGGGTCACAG	GGTTGTG	CAGCCCTTGCCCTGACTGATTGGCAGCCGATTGAACAGCCTATGCGAGC	TTCCTG
CGGGTTTGT	GTGTGGGGTTGA	GCCTGTG	TGCTCCTATGAACAGTACTTCGGTCCCGGCCACGAGCTCACGGTTTTAG	GTAAGA

Fig. 6 Flanking sequence homologies among *J* regions both 5' and 3' (see text); homologies are underlined.

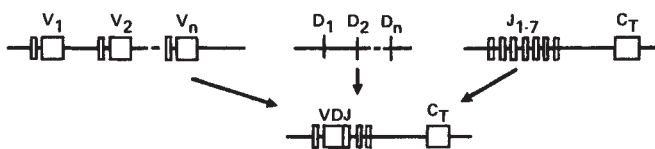


Fig. 7 Model of 2B4 T-cell receptor gene rearrangement. Variable (V), diversity (D) and joining (J) region elements all appear to contribute to the formation of the 2B4 gene examined here.

perhaps to allow independent regulation of rearrangement in T and B cells. Another possibility is that there is no functional difference between the flanking sequences of the T-cell *J* regions and those of immunoglobulins; this would account for the non-functional *D-J* joining found in some T-cell clones²⁵ and would suggest that the usual B cell/T cell specificity of rearrangement is dependent on other signals. No immunoglobulin light-chain rearrangement has been observed in T cells and we have not seen any rearrangement of the T-cell locus discussed here in three different B-cell lymphoma lines, nor in a macrophage line.

Figure 5 illustrates the homology between *J* regions of the cluster described here in the potential coding residues. As with *J* regions in any specific immunoglobulin family, all except the pseudo-*J* are highly homologous. Note also that the previously identified (from cDNA clones) 86T1/86T5 *J* region⁹ is not within this cluster, nor is another *J* region found in a cDNA clones from a concanavalin A-stimulated spleen cell library (unpublished results). These and other *J_T* regions appear to reside in a second cluster 5' to the one examined here⁴².

Implications for antigen recognition

The T-cell gene studied here bears a remarkable resemblance to those of immunoglobulins in amino acid sequence, patterns of rearrangement and the sizes and positions of the different gene segments. Even the flanking sequences relevant to rearrangement differ only slightly. These similarities must reflect the common origin of these genes in evolution as well as the strength of the selective pressures involved. Also interesting is the complexity of the rearrangements (see Fig. 7); the formation of the variable-region exon requires as many gene segments (*V*, *D* and *J*) as the most complex of the immunoglobulins. This is in contrast to the expectation that the T-cell receptor should represent a simpler type of gene (perhaps like light-chain immunoglobulins) because cell-mediated immunity may have arisen earlier in evolution than humoral (antibody-mediated) immunity. Instead, it seems that the T-cell gene is at least as

complex as the most elaborate of the immunoglobulin genes and that T-cell recognition of antigen and MHC may involve a range of specificities equivalent to that of immunoglobulins. It is also interesting to note that the '11-22' rule²¹ which in B-cell heavy chains may only permit *V* and *J* segments to join to *D* (and not to each other) does not exclude direct *V-J* joining in the example seen here. If such direct joining is possible, it would provide additional flexibility in the formation of the variable region of this molecule.

The similarities to immunoglobulins noted above seem to place considerable constraints on the structure of the second chain of the receptor heterodimer. The intertwining of the heavy- and light-chain polypeptides with each other in immunoglobulins as defined by X-ray crystallography^{32,33} makes it unlikely that one chain could be so similar to immunoglobulin and the other chain very different. This idea is reinforced by the peptide mapping data indicating variable and constant portions of both chains³⁴⁻³⁶. Therefore, one would expect the second chain to be very similar to the one described here and that the combining site formed must resemble that of immunoglobulin. Indeed, even the possible hypervariable and framework residues seen in the comparison with 86T1 and p2B4.71 (Fig. 1) fall into almost the same positions as those of immunoglobulin. This favours the one-receptor model of antigen-MHC recognition, but introduces the unexpected paradox of how to account for all the additional requirements of T-cell recognition and thymic learning in a molecule that is as yet indistinguishable from immunoglobulins. One possibility is that a low-affinity interaction with self-MHC occurs in the thymus which eliminates T cells which either cannot bind to self-MHC at all, or bind too tightly. Evidence has been obtained for the existence of highly self-MHC-reactive cells being present in, but never leaving the thymus (B. Benaceraf, personal communication). Perhaps only those cells with a low affinity for self-MHC can emerge from the thymus and are available to recognize antigen-MHC complexes, which they presumably do at some higher affinity. Alternatively, the receptor molecule could be largely used in binding antigen while the MHC interaction depends more on accessory molecules such as L3T4 in helper T cells³⁷ and Lys 2,3 in cytotoxic T cells³⁸. The availability of cloned probes and detailed structural data should considerably aid experiments designed to test this and other models.

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Kinked DNA in crystalline complex with *EcoRI* endonuclease

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The 3 Å electron density map of a co-crystalline recognition complex between EcoRI endonuclease and the oligonucleotide TCGCGAATTCGCG reveals that a tight, complementary interface between the enzyme and the major groove of the DNA is the major determinant of sequence specificity. The DNA contains a torsional kink and other departures from the B conformation which unwind the DNA and thereby widen the major groove in the recognition site.

THE interactions between proteins and specific DNA sequences are key regulatory events in the control of many biological processes. A full understanding of the recognition mechanisms which determine these interactions requires detailed knowledge of the structure of representative DNA-protein interfaces. This knowledge may also reveal general recognition rules which could govern a broad range of DNA-protein interactions. Recently, the crystal structures of several proteins which recognize specific sites on DNA have been solved¹⁻⁵. In the absence of DNA-protein co-crystals of high quality, these protein structures alone have provided the basis for extensive model building and speculation concerning the appearance of the recognition complexes¹⁻⁸. However, these studies are limited by the necessary assumption that significant conformational changes do not occur in either the protein or the DNA. Similarly, crystallographic studies on B-DNA have revealed detailed information on the structure of DNA in the absence of protein⁹⁻¹⁴. We report here our preliminary observations of the first sequence-specific DNA-protein complex to be visualized by X-ray diffraction methods: *EcoRI* endonuclease complexed with the synthetic oligonucleotide TCGCGAATTCGCG (*EcoRI* site underlined).

Solution of the structure

EcoRI endonuclease and TCGCGAATTCGCG co-crystallize in space group P321 with one 32,000-molecular weight enzyme subunit and one strand of DNA in the asymmetric unit, as reported previously¹⁵. The crystals were grown in the absence of Mg²⁺, which is required for cleavage of the DNA. Data were collected by oscillation film photography as described previously¹⁵ on the native crystal and on platinum and mercury derivatives. The platinum derivative was obtained by soaking crystals in 10 mM *cis*-(NH₃)₂PtCl₂, dissolved in 40 mM bis-tris-propane (BTP), pH 7.0, plus a mixture of 11% w/v polyethylene glycol (PEG) 6000 and 5% w/v PEG 400 for 7 days at 25 °C. The Hg derivative was similarly obtained by soaking crystals in 5 mM Hg(SCH₂CH₂NH₃)₂, dissolved in 40 mM BTP, pH 7.0, plus 16% w/v PEG 6000 for 3.5 days at 25 °C. The heavy atom locations were independently determined by difference Patterson methods for both derivatives. (Even though the platinum compound is a chemotherapeutic agent which appears to act at the DNA level, the location of the major and minor sites are found on the surface of the protein, well away from the DNA.) The Cullis *R* factors for the Pt and Hg derivatives to 3.5 Å are 49.2% and 52.2%, respectively.

A multiple isomorphous replacement (MIR) electron density map was calculated from these two derivatives to 5 Å resolution. In addition, Wang's iterative single isomorphous replacement (ISIR) method (B.-C.W., in preparation) was independently applied to the Pt and Hg data. This procedure identifies protein and solvent regions in a SIR electron density map and uses this

information to resolve the SIR phase ambiguity. The general features, such as the solvent regions and the molecular outline, were similar in all three electron density maps; however, the MIR and Hg-ISIR maps contained significant amounts of noise, while the Pt-ISIR map was very clear. We suspect that the noise is associated with non-isomorphism in the Hg derivative (even the Pt derivative was not strictly isomorphous beyond 3.5 Å). We therefore used the ISIR method to resolve the phase ambiguity in the 3.5 Å Pt SIR phases. The ISIR method was also used to extend the phase information in successive stages to 3.2 and 3.0 Å resolution. The mean figures of merit for the 3.5 and 3.0 Å phases are 0.78 and 0.79, respectively. Electron density maps calculated with the 3.5 and 3.0 Å Pt-ISIR phases were very similar apart from the expected greater detail in the higher resolution map. Both maps showed clearly recognizable features, including the α -helix seen in Fig. 1. The enantiomorph was chosen so that α -helices were right handed.

The DNA was easily located in the 3.0 Å map and coordinates were obtained for the centroids of all 12 phosphate moieties, the 13 sugars and 12 of the bases. These coordinates enabled us to calculate the general DNA conformational parameters via the helix determination procedures developed in conjunction with the ApU and GpC structures¹⁶ as well as to construct Figs 2 and 3.

Structural features

Inspection of the Pt-ISIR electron density map revealed several striking features which were clear before a complete tracing of the polypeptide chain and fitting of molecular models. These broad features are discussed below.

The DNA-*EcoRI* endonuclease complex is a symmetrical globular structure ~50 Å across (Fig. 2). The molecular boun-

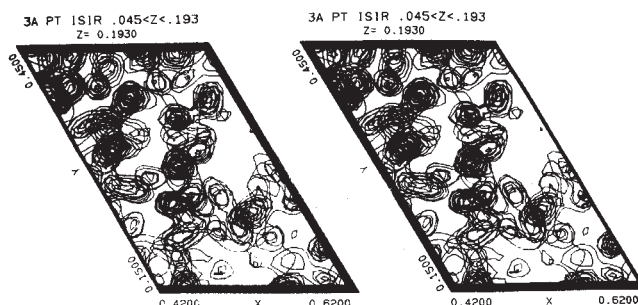


Fig. 1 A representative α -helix. A stereo pair showing a portion of the 3.0 Å ISIR electron density map illustrating the general quality of the map. An α -helix runs vertically up the page (this is not the α -helix discussed in the text which interacts with the DNA).

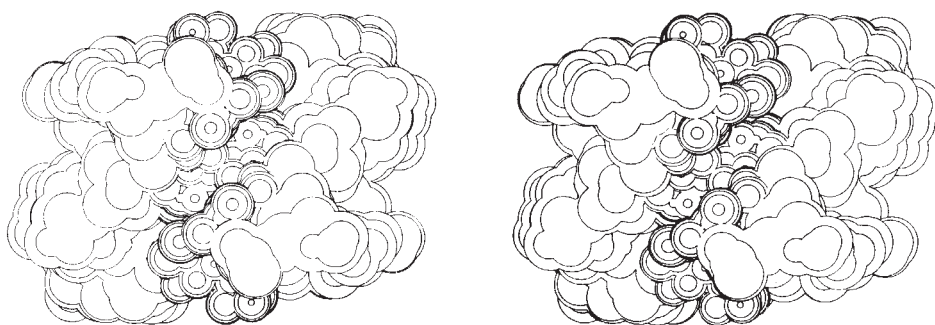


Fig. 2 The DNA-*EcoRI* endonuclease complex. This schematic representation of the surface of the DNA-protein complex was constructed by superimposing spheres on the electron density map so that their surfaces followed the molecular boundary. The DNA is shaded more darkly than the protein. The complex is viewed from the solvent region, looking down the crystallographic 2-fold symmetry axis. The DNA is clearly embedded in the protein and the active sites for recognition and hydrolysis are underneath the DNA.

dary clearly encloses a well defined complex consisting of two enzyme subunits and one double helix. The two protein subunits and the two strands of DNA are related by a common crystallographic axis of 2-fold symmetry, as expected from the contents of the asymmetric unit¹⁵.

The packing of these complexes within the crystalline lattice is typical of that generally seen in macromolecular crystals, that is, a limited number of fortuitous interactions between symmetry-related complexes. One of these is a DNA-DNA interaction in which symmetry-related double helices are packed end to end, forming a continuous rod of DNA parallel to the *c*-axis. This is one of the common modes of packing found in crystals containing only DNA and probably contributes to the relatively strong diffraction pattern of this particular crystalline form of *EcoRI* endonuclease. The unpaired thymine moieties at each end of a double helix appear to be in contact with thymine residues from symmetry-related double helices.

The DNA conformation departs from the classical B-motif (Fig. 3). The 'distortions' are not uniformly distributed over the entire DNA molecule; rather they are concentrated in three abrupt transitions termed kinks. These kinks create four blocks of three base pairs each:



where the kinks are indicated by dashes. These features arise from systematic shifts in the coordinates of all the phosphate, sugar and base moieties in each intervening 3-base pair (bp) segment, that is, they represent local adjustments to net displacements of entire blocks of double helical DNA. These kinks are conceptually related to the model structures originally predicted by Crick and Klug¹⁷, although they differ significantly in detail. Additional kinked models were proposed by Sobell *et al.*^{18,19} and by Zhurkin *et al.*²⁰.

We suggest a flexible nomenclature for the DNA conformation because subsequent DNA-protein structures will probably reveal additional kinks and other novel DNA features. We refer to the overall DNA conformation as 'neo RI-DNA'. The specific context of neo relates to a DNA conformation which is stabilized by the binding of a recognition protein.

The left-most block will be referred to as the terminal or CGC block and the next block as the central or GAA block. The two blocks on the right (TTC and GCG) are identical to the respective left blocks because of the 2-fold symmetry axis.

We designate the transition between the symmetry-related central blocks (GAA-TTC) as the neo-1 kink and that between the terminal and central blocks (CGC-GAA) as the neo-2 kink. Neo-2 kinks appear twice within the structure (CGC-GAA and TTC-GCG) and both are identical by virtue of the crystallographic axis of 2-fold symmetry.

When the helical symmetry within each block was determined by analysing the geometrical relationships of the centroids of the phosphate and internal deoxyribose moieties according to the procedure of Rosenberg *et al.*¹⁶, B-type parameters were found for the central block: 10.3 residues per turn with a helical displacement of 3.2 Å. The values for the terminal block appear more like those usually associated with A-DNA: 11.3 residues

per turn and 2.7 Å per residue. The location of the helix axes and the apparent tilt of the bases with respect to these axes are also suggestive of B- and A-DNA in the two respective blocks.

We emphasize that we are not claiming that the blocks between kinks are identical to either 'classical' A- or B-DNA. Indeed, these designations must be considered tentative until our analysis has reached the point that sugar pucker and other detailed conformational parameters can be determined unambiguously. We also suspect that relatively smaller structural departures from exact helical symmetry will be noted within the blocks once the structure is carefully refined.

Comparison with other DNA structures

The DNA in our co-crystal is basically the dodecamer studied extensively by Dickerson and colleagues^{9-14,21} with the addition of a 5'-thymidylate residue. The kinks described here differ significantly from the features reported by Dickerson *et al.*, suggesting that the neo RI-DNA structure is not stable in the absence of the enzyme. This does not exclude the possibility that the neo RI conformation exists transiently due to thermal fluctuations nor does it exclude the possibility that these transient states are kinetic intermediates of complex formation.

Dickerson's dodecamer structure also possesses A-like features in the region we have described as the CGC block¹⁰. This led Dickerson and Drew to suggest that *EcoRI* sites are B-like within the canonical hexanucleotide flanked by A-like regions¹⁰. Our structure appears to confirm this suggestion and to indicate that this trend is amplified in the protein-DNA complex.

The 10.3 bp per turn helical repeat of the central blocks is within the values of 10.0 to 10.6 bp per turn reported for DNA in solution^{22,23}, bound on crystalline surfaces^{24,25} and in nucleosomes^{26,27}.

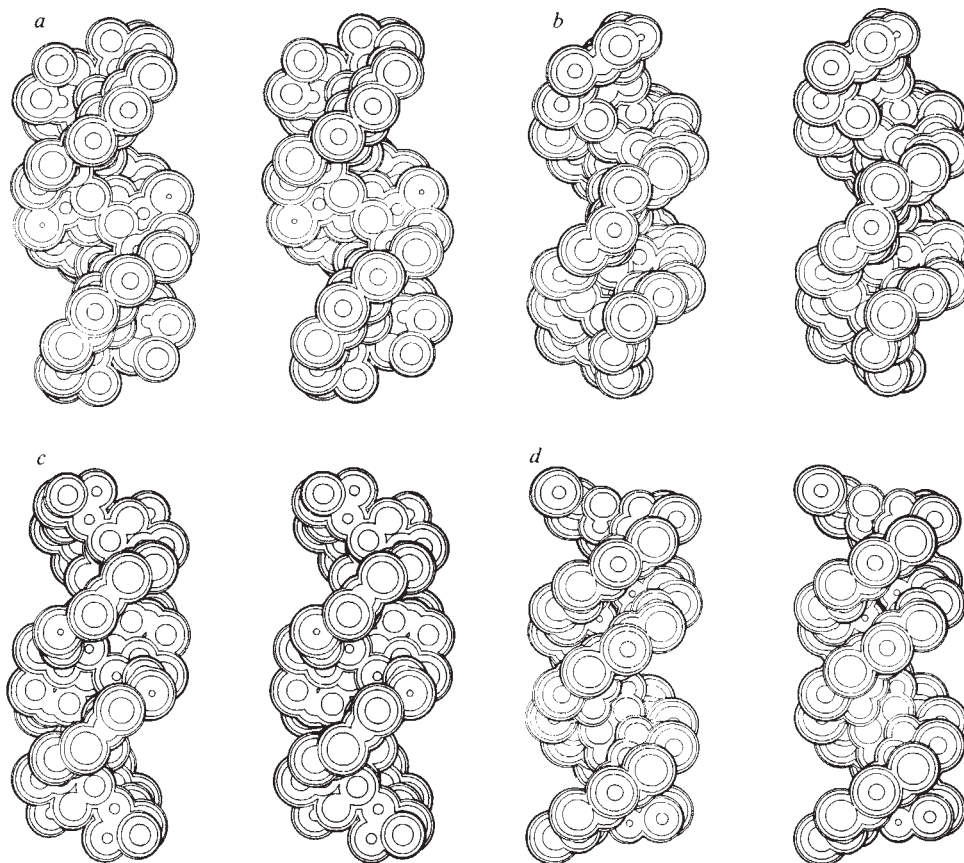
Nature of the kinks

The neo-1 kink, at the centre of Fig. 2, enlarges the grooves, thereby facilitating access by the protein to the bases. This kink is a prominent feature of Fig. 3b, where the backbone can be seen to 'jog' upward in the centre of the figure. The displacement increases the distance between those segments of DNA backbone which straddle the kink by ~4 Å. This is achieved by a systematic unwinding of the entire DNA molecule by ~25°, that is, the neo-1 kink is primarily a torsional dislocation of the double helix.

The neo-1 kink introduces a small bend in the DNA such that the helices of the symmetry-related central blocks are inclined at an angle of ~12° to each other. (This value is preliminary and it is anticipated that model fitting and refinement will result in adjustments to its value.) The bend is towards the minor groove, as suggested by Crick and Klug¹⁷.

The neo-2 kink is different in several respects from neo-1. The neo-1 possesses 2-fold symmetry while the neo-2 kink is asymmetrical. The neo-2 kink does not involve a dramatic unwinding of the DNA; however, it introduces a larger bend of ~23° between the helix axes. This bend includes rolling motion towards the minor groove. The neo-2 kink may also represent an A-B junction.

Fig. 3 The neo RI-DNA conformation. *a*, A stereo representation of the portion of the electron density corresponding to the DNA produced by placing spheres at the approximate positions of the phosphate, deoxyribose and base moieties (radii of 4, 3.5 and 3.5 Å, respectively). The view is from within the protein, towards the major groove where the sequence-specific recognition contacts are concentrated. The two parallel segments of DNA backbone running across the front of the figure are buried in clefts in the enzyme and include the phosphodiester linkages which would be cleaved in the presence of Mg^{2+} . *b*, The DNA rotated by 90° about the average helix axis. The neo-1 kink, which is between the two central blocks, can be seen in the centre. Note also the expansion of the major groove (which opens towards the left in the upper portion of the figure). *c*, The DNA rotated by 180° from that shown in *a*, that is, viewed from the solvent region as in Fig. 2. Kinks between the terminal blocks of 3 bp and the middle blocks can be noted in the backbone segments closest to the viewer. They are of the type described as neo-2 in the text. *d*, A similar representation of the B-DNA model which Arnott and co-workers³⁰ derived from fibre diffraction data.



Recent crystallographic observations of DNA structural variability *in vitro* have led to suggestions that DNA *in vivo* is structurally heterogeneous, and that structural variations may be associated with sequence-specific DNA-protein recognition^{10,28}. The structural results include major departures from B-DNA, such as Z-DNA²⁸⁻³¹ as well as relatively smaller local variations within the B-motif^{10,14,32,33}. The association between protein recognition and altered DNA conformation has been enhanced by the observation that DNA sequences which have the potential to form Z-DNA can be found within certain eukaryotic regulatory elements^{34,35}.

The kinks described here represent additional experimentally observed elements of DNA structural heterogeneity which are clearly associated with the binding of a recognition protein. The neo-1 kink is an essential element of the recognition event itself because the symmetry-paired α -helices (see below) cannot fit into the major groove unless that groove has been expanded via the neo-1 kink. This does not exclude an additional involvement of the kinks, especially the neo-2 kink, in the strand scission event also. This raises the expectation that DNA-protein recognition in general will turn out to be associated with specific deviations from the average B-double helix. The neo-1 and neo-2 kinks are probably general structural features that will be found in other DNA recognition systems. However, these generalizations cannot yet be established since this is the first X-ray structure of DNA in intimate association with a sequence-specific recognition protein.

DNA-protein interaction

The major groove of the recognition hexanucleotide (GAATTC) appears to be filled with protein, forming a large, complementary interface. All the base-amino acid interactions appear to be located in the major groove of the DNA, while the edges of the bases which are exposed in the minor groove are open to the solvent. Thus, the primary determinant of the recognition specificity is the direct interaction of complementary surfaces in the major groove. However, additional features of this structure

raise the possibility of a second, indirect mechanism whereby sequences flanking the canonical hexanucleotide modulate the binding (see below).

A major component of the DNA-protein interaction is an α -helix which makes an angle of $\sim 60^\circ$ with the average DNA helix axis. There are two such α -helical structures, side by side, related by the crystallographic 2-fold symmetry axis. The polypeptide chain turns sharply at the end of each α -helix, leaving the amino acid residues at the end of the helix, in the bend and those in the adjacent stretch of chain in close proximity to the DNA.

Two symmetry-related pairs of amino acid side chains appear to be in contact with symmetry-related pairs of sequential adenine residues. These amino acid side chains are located in the α -helix-bend motifs described above, although each of the two physically adjacent side chains originates from a different protein subunit.

Flanking the recognition interface are two symmetry-related DNA backbone segments, those nearest the viewer in Fig. 3*a*, which are deeply buried in symmetrical clefts in the enzyme. These clefts extend from the bottom of the wide semi-cylindrical depression which contains the DNA, giving the protein a 'threaded' appearance. The buried backbone segments include the scissile phosphodiester bonds.

While the two 'arms' of protein enveloping the DNA from the upper left and lower right of Fig. 2 appear to originate in the underlying subunits, they might alternatively originate from symmetry-related molecules which are nearby due to crystal packing. We favour the interpretation that they are part of the underlying subunits because the electron density maps show extensive continuity with the underlying electron density and very limited continuity with the nearby, symmetry-related electron density. Furthermore, we have a provisional tracing of the polypeptide chain in this region in which each arm is a loop of protein which emerges from the underlying region. However, we emphasize that we cannot entirely exclude the alternative until we have an unambiguous tracing of the entire polypeptide chain.

If these arms do extend from the underlying protein, this would necessitate a conformational change in the enzyme during the complete catalytic cycle because the DNA could not get past the arms and enter the active site unless something moves. Such a conformational change has been implied by some of our biochemical results (ref. 36 and L.J.-J. *et al.*, unpublished). The conformational change could arise from two possibilities which are not mutually exclusive: mobility of the arms themselves and quaternary changes in which the two subunits in the dimer move with respect to each other.

Comparisons with inferred contact points

The DNA contact points described above show both similarities and differences when compared with the contacts inferred by Lu *et al.*³⁷ from the results of alkylation interference and protection experiments. They suggested contacts in the major groove (at the recognition guanine N⁷ position) as well as phosphate contacts in good general agreement with our structure. Lu *et al.* also made two observations regarding the N³ position of the central adenine, which is in the minor groove. This site was protected from dimethylsulphate by endonuclease, and prior methylation at this point blocked subsequent binding of the enzyme. They suggested that *EcoRI* endonuclease is in physical contact with the central adenine at N³ (however, they also indicated that other interpretations were possible). We do not see any interaction between the protein and DNA at that position. This methylation site is in the region of the DNA most affected by the neo-I kink and base-base steric effects could be responsible for these methylation results. Lu *et al.* also observed that while the N³ of the adjacent adenine was protected by the endonuclease, prior methylation at this second point did not block subsequent binding of the enzyme. The factors responsible for this result are unknown, although we do not see any minor groove DNA-protein interactions there either. We therefore conclude that while alkylation protection and interference data can be used to infer a good low resolution indication of the location of the DNA-protein contacts, they cannot be reliably used to infer the precise points of DNA-protein interaction.

Another approach used to identify contacts on the DNA is based on the hydrogen bonding degeneracy rules of Seeman *et al.*³⁸⁻⁴⁰. In *EcoRI* endonuclease such degeneracy can be induced by conditions of elevated pH (8-9.5), Mn²⁺, low ionic strength and by the addition of organic compounds such as glycerol or ethylene glycol⁴¹⁻⁴⁵. This has been called the *EcoRI** reaction⁴¹ and the non-canonical sites so recognized are referred to as *EcoRI** sites. We showed that hierarchies of base preferences could be discerned by qualitative estimations of the rate of cleavage at *EcoRI** sites in plasmid DNA molecules. The observed hierarchies are uniquely predicted by a recognition model which assumes two major groove hydrogen bonds per base pair in standard conditions⁴⁰. One or more of these are randomly replaced by a water-DNA hydrogen bond in *EcoRI** conditions. The position of any *EcoRI** sequence within the appropriate hierarchy is primarily determined by the number of remaining protein-DNA hydrogen bonds⁴⁰. Woodbury *et al.*³⁹ reached similar conclusions from a somewhat different perspective. The major groove recognition points inferred from these analyses are in excellent agreement with our X-ray structure. Thus, degeneracy analysis appears to be a promising method for the study of DNA-protein interactions.

Flanking sequence effect

Thomas and Davis⁴⁶ established that DNA sequences flanking the recognition site influence the hydrolysis rate by approximately an order of magnitude. These results have been confirmed and extended⁴⁷⁻⁴⁹. Two hypotheses (which are not mutually exclusive) can be advanced to explain these data: (1) Contacts occur between the enzyme and DNA bases outside the canonical hexanucleotide; (2) the conformational free energy of the neo-2 kink and/or of the terminal block depends on the flanking base sequence.

We have seen no significant interactions between *EcoRI* endonuclease and bases outside the canonical hexanucleotide. However, we must again emphasize that this cannot be stated unequivocally until all relevant side chains have been located and their positions refined. We do see substantial interactions between the protein and DNA backbone segments which extend beyond the canonical hexanucleotide. These observations are consistent with the latter hypothesis.

This raises the possibility that *EcoRI* endonuclease interacts with DNA in two sequence-dependent ways: (1) A direct specificity interaction with the central hexanucleotide which creates the precise requirement for the *EcoRI* sequence, GAATTC; (2) an indirect interaction with sequence 'selectivity' which subtly modulates the binding energy as a function of base sequence within the regions immediately flanking the *EcoRI* site. Sequence specificity is mediated by physical contact, such as hydrogen bonds, between the protein and DNA bases. Sequence selectivity may be indirectly mediated by sequence-dependent DNA conformational forces.

Conclusions

The following observations include those which we feel are most significant: (1) The direct sequence-specific DNA-protein interactions involve the major groove of the DNA in a tight, complementary interface with *EcoRI* endonuclease; (2) minor groove contacts do not appear to be significant determinants of sequence specificity in this case; (3) the DNA conformation in the recognition complex is a departure from the B-motif; (4) the torsional neo-I kink is required for the protein to fit into the major groove of the DNA; (5) kinks are now experimentally observed elements of DNA structural heterogeneity which may have a general role in DNA-protein recognition.

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LETTERS TO NATURE

Reverse stellar evolution, quasars and low-mass X-ray binaries

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Recently, Mathews has proposed¹ a mechanism for the mass supply to a black hole in the centre of a quasar (see also ref. 2). According to this proposal the X rays from the central source penetrate the photosphere surrounding low-mass stars. The absorbed energy is distributed over the stellar interior by convection, and the star expands rapidly. Its outer layers are then stripped by the radiation field of the central source. There are two major uncertainties in this model of reversed evolution; the depth to which the X rays impinging on a low-mass star can penetrate before they are absorbed and the inward transport of the energy flux. The crucial hypothesis of Mathews is that the X rays are absorbed within the convection zone. We now test this hypothesis by considering the effects of X-ray absorption in the secondaries of low-mass X-ray binaries. We conclude that reverse evolution does not occur.

In low-mass X-ray binaries a late dwarf transfers matter by Roche-lobe overflow to a neutron star through an accretion disk³. As generally no X-ray eclipses in these systems are observed, it has been concluded that the secondaries are shielded from the X rays of the neutron star by the accretion disk⁴. Optical studies have shown that this shielding is not complete. Orbital variations in the optical, due to X-ray heating of the surface of the secondary facing the neutron star, have been detected in Sco X-1, 4U2129+47 and 4U/MXB1735–44 (ref. 5). In Sco X-1 and 4U1735–44 part of these variations may be due to heating of a bulge in the accretion disk⁵. The recently discovered sharp X-ray eclipses in MXB1659–29 (ref. 6) show that the secondary in this system intercepts X rays from the neutron star. Optical pulses have been detected in 4U1626–67 at a sidelobe frequency to that of the X-ray pulses and shown to arise from X-ray reprocessing on the secondary. The strength of the optical pulses shows that a large fraction of the surface of the secondary facing the neutron star is exposed to the X rays⁷. The importance of these observations for the present discussion is that they show that the secondaries in low-mass X-ray binaries are exposed to X-ray irradiation, and that reverse evolution—if viable—should occur in them. In particular, we note that a main sequence companion star to 4U1626–67 would be $\sim 0.08 M_{\odot}$ (ref. 7), and therefore precisely the kind of red dwarf considered by Mathews¹.

We will compare the time scale of the evolution of an X-ray binary undergoing reverse stellar evolution with the Kelvin–Helmholtz time scale of the mass-losing secondary

$$\tau_{KH} = \frac{GM^2}{RL} \approx 3 \times 10^7 \text{ yr} \left(\frac{M_{\odot}}{M} \right)^2 \quad (1)$$

Here M and R are the mass and radius of the secondary, respectively. To obtain the last equality we have used the mass–radius and mass–luminosity relations for the main sequence $R \propto M$ and $L \propto M^3$. For fully convective low-mass stars the Kelvin–Helmholtz time scale is of the order of 10^9 yr.

Let us suppose that the X-ray energy flux were transported

inwards to heat the interior directly. The star would then adjust its structure on a time scale given by

$$\tau_X = \tau_{KH} \frac{L}{L_a} \quad (2)$$

where $L_a (\gg L)$ is the absorbed luminosity. A single star in the X-radiation field of a quasar would expand on the time scale given by equation (2), which is much shorter than the Kelvin–Helmholtz time scale. In a low-mass binary the expansion would lead to mass-transfer on the expansion time scale τ_X .

To estimate the relevant time scales we have to determine the amount of absorbed X-ray energy, which in the binary can be written

$$L_a = f \frac{\pi R^2}{4\pi a^2} L_X \approx \frac{f}{4} \left(\frac{R}{a} \right)^2 \frac{L_X}{L_{\text{Edd}}} 1.8 \times 10^{38} \text{ erg s}^{-1} \quad (3)$$

Here a is the semi-major axis of the binary orbit, L_X is the X-ray luminosity and L_{Edd} is the Eddington luminosity of a $1.4 M_{\odot}$ neutron star, equal to $1.8 \times 10^{38} \text{ erg s}^{-1}$. f is the product of a correction factor to the rough estimate $(R/a)^2/4$ of the spatial angle subtended by the secondary as seen from the neutron star, the fraction of this spatial angle that is not shielded by the accretion disk, and the fraction of the X rays impinging on the surface that penetrate into the stellar interior. Assuming with Mathews that approximately half of the X-ray flux penetrates deeply, we find typical values for f between a fifth and a tenth. (R/a) is a slowly varying function of the secondary mass, well approximated by⁸

$$\frac{R}{a} \approx 0.46 \left(\frac{M}{M + M_{\text{ns}}} \right)^{1/3} \quad (4)$$

Combining equations (1)–(4) we get

$$\tau_X \approx \frac{1.3 \times 10^4}{f} \text{ yr} \frac{M}{M_{\odot}} \left(\frac{M + M_{\text{ns}}}{M} \right)^{2/3} \frac{L_{\text{Edd}}}{L_X} \quad (5)$$

If reverse evolution could operate, the following would happen in a low-mass X-ray binary. Once the mass transfer starts, the X rays will penetrate into the secondary and cause it to expand. The expansion will increase the mass transfer, and hence cause a higher X-ray luminosity. Thus the mass transfer and the X-ray luminosity grow exponentially, until the Eddington luminosity is reached, or until the mass flow prevents the radiation produced by the accretion process reaching the convection zone of the secondary. After this the evolution proceeds linearly on the time scale given by equation (5), that is shorter than $\sim 10^5$ yr. The initial exponential growth of the mass transfer rate occurs in the time $\tau_X h/R$ needed by the secondary to expand an atmospheric pressure scale-height h outside its Roche lobe. This is within $\sim 10^4$ yr, even for low-luminosity X-ray binaries ($L_X \approx 10^{36} \text{ erg s}^{-1}$).

The consequences for our Galaxy are then that most low-mass X-ray binaries should be at the Eddington limit, and presumably many of them would show strong absorption effects in the X rays. This is not supported by the observations. Only if the shielding of the secondary by the accretion disk and the scattering of the hard X rays reduces f below $\sim 10^{-4}$ could reverse evolution be compatible with the observations.

In addition, if low-mass binaries were undergoing reverse evolution their average life time would be of the order of 10^5 yr

only. The impossibility of such short life times is particularly clear in globular clusters. The number of bright low-mass X-ray binaries observed in each of the densest globular clusters is of order unity⁹. For an average life time of τ yr this means that a system has to be formed in a typical dense cluster every $1/\tau$ yr. The formation rate¹⁰ as calculated from the number of close encounters between compact remnants and main sequence stars in the cluster is in good agreement with an average life time of the low-mass X-ray binary of $\sim 10^9$ yr, but not with a life time of 10^5 yr.

We note that a secondary in a low-mass X-ray binary receives an X-ray flux $\geq 10^4$ times that of a low-mass star in the X-ray radiation field of a quasar. The X-ray flux received by the latter is more comparable with that received by a secondary in a cataclysmic variable. There is no evidence for reverse evolution in cataclysmic variables.

We have offered empirical evidence that reverse evolution does not occur. But we must not overlook the severe theoretical problems with this idea. An intense flux of incident radiation would tend to quench convection in the outer layers; even if free energy were somehow available to maintain the star in a well-mixed (constant entropy) state, thermodynamical arguments would still restrict the efficiency of inward energy transport against the temperature gradient.

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The nature of low-frequency cutoffs in the radio emission spectra of pulsars

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Low-frequency cutoffs were first observed in the radiation spectra of several pulsars about a decade ago¹. The detailed study of the low-frequency ($\omega \leq 100$ MHz) radio emission spectra of more than 50 pulsars^{2–7} has shown that practically all pulsars observed have low-frequency cutoffs in their spectra. In most pulsars the spectral cutoffs are an inherent feature of pulsar emission and are obviously associated with the emission mechanism⁸. The most favoured hypothesis at present is that the radio emission of pulsars is a curvature radiation of charged plasma bunches that move, with a Lorentz factor of $\Gamma \approx 10^3$, practically along curved magnetic field lines^{9,10}. The kinetic energy of plasma bunches is comparable with the brightness temperature of radio emission measured in energy units. Hence, the reabsorption of the curvature radiation by the radiating plasma bunches themselves may be essential for the formation of the radio emission spectra of pulsars. Here we show that this radiation reabsorption may be responsible for low-frequency cutoffs in the pulsar radio emission spectra.

The curvature radiation of a system of charged ultrarelativistic particles ejected from the polar cap of a pulsar with a dipole magnetic field has been discussed in detail elsewhere¹¹, taking into account the emission reabsorption by radiating particles. The spectral density of the curvature radiation flux $F(\vartheta_K, \omega)$ was derived for the direction forming an angle ϑ_K with the

magnetic axis at a frequency ω . Following ref. 11 we consider the case of a monoenergetic distribution of ejected particles and the power dependence of their density at the pulsar surface on the distance d to the pulsar's magnetic axis:

$$f(\Gamma, \vartheta_0) = K_0 \delta(\Gamma - \Gamma_0) \left(\frac{\lambda}{\vartheta_0} \right)^\beta \Theta[\vartheta_0 - \lambda] \Theta[\hat{\vartheta}_0 - \vartheta_0] \quad (1)$$

where $f(\Gamma, \vartheta_0)$ is the distribution function of ejected particles, K_0 and β are constants, $\vartheta_0 = \arcsin(d/R)$ is the angular co-ordinate of points on the pulsar surface, measured from its magnetic axis, $\Theta[x]$ is the step function equal to unity for $x \geq 0$ and to zero for $x < 0$, and $\hat{\vartheta}_0 \approx (\Omega R/c)^{1/2}$ is the angular radius of the polar cap of the pulsar, separating the open magnetic field lines, along which particles are ejected ($\vartheta_0 < \hat{\vartheta}_0$), from the closed lines, along which no plasma ejection occurs ($\vartheta_0 > \hat{\vartheta}_0$); $\lambda \approx \hat{\vartheta}_0^2$; Ω is the angular rotation velocity of the pulsar and R is its radius.

The observed average flux of the curvature radiation at the frequency ω is given by

$$\overline{F(\omega)} = \int_0^{2\pi/\Omega} F(\vartheta_K(t), \omega) \frac{c}{(2\pi)} dt \quad (2)$$

where ϑ_K versus t is

$$\cos \vartheta_K = \cos \vartheta_{\Omega B} \cos \vartheta_{\Omega K} + \sin \vartheta_{\Omega B} \sin \vartheta_{\Omega K} \sin \Omega t \quad (3)$$

$\vartheta_{\Omega B}$ is the angle between the rotation axis and the magnetic axis, and $\vartheta_{\Omega K}$ is the angle between the rotation axis and the direction towards the observer.

By substituting into equation (2) the expression $F(\vartheta_K, \omega)$ given for the respective frequency intervals by (76)–(79) (ref. 11), we may obtain the integral flux $\overline{F(\omega)}$. In a partial case $\vartheta_K^{\min} = |\vartheta_{\Omega B} - \vartheta_{\Omega K}| = 0$, when in the pulsar rotation process the centre of its curvature radiation beam may coincide with the direction towards the observer, the expression for $\overline{F(\omega)}$ is readily integrable.

The curvature radiation spectrum $\overline{F(\omega)}$ as a function of frequency dependence, for $\vartheta_{\Omega B} \sim 1$, $\vartheta_K^{\min} = 0$ and $\beta > 2$, is written as (see Fig. 1):

$$\overline{F(\omega)} \propto \begin{cases} \omega^{-(\beta-2)} & \text{at } \omega_B \ll \omega \leq \frac{9}{8} \frac{c}{R} \Gamma_0^3 \hat{\vartheta}_0, \\ \omega^{-4(\beta-2)/(6+\beta)} & \text{at } \omega_m \ll \omega < \omega_B \\ \omega^{(6\beta-8)/(1+3\beta)} & \text{at } \omega \ll \omega_m, \end{cases} \quad (4)$$

where

$$\omega_B = \left[\frac{3}{4} \left(\frac{2}{3} \right)^{2/3} \frac{(\beta-2) e^2 \lambda^{\beta-2} \dot{N}}{mc R^2 \Gamma_0} \left(\frac{8 R}{9 c} \frac{1}{\Gamma_0^3} \right)^{-\beta} \right]^{1/(2+\beta)} \quad (5)$$

$$\omega_m = \omega_B \hat{\vartheta}_0^{[16/3(2+\beta)]} \quad (6)$$

where $\dot{N}$ is the number of particles ejected from a pulsar per unit time.

Both a break in the spectrum at ω_B and its low-frequency cutoff at the frequencies below ω_m result from the curvature radiation reabsorption by radiating particles as such. In the case considered, two characteristic frequencies appear in the radiation spectrum due to the non-uniformity and anisotropy of a curvature radiation source.

In the case of radio emission from pulsars, particles should be regarded as bunches of electron–positron plasma. Then in equations (5) and (6) the charge Q , mass M and the flux of

Table 1 Values of curvature radiation flux as a function of frequency dependence

	$\frac{6\beta-8}{1+3\beta}$	$\frac{4(\beta-2)}{6+\beta}$	$(\beta-2)$	ω_m (MHz)	ω_B (GHz)
$\beta = 3$	-1	0.44	1	75	10
$\beta = 4$	-1.23	0.8	2	134	8
$\beta = 5$	-1.37	1.09	3	197	6.5

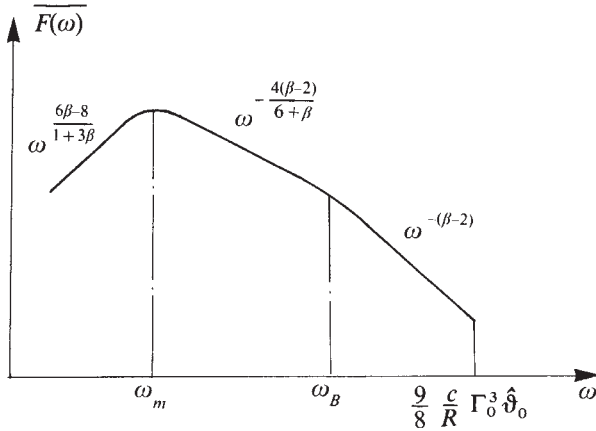


Fig. 1 The curvature radiation spectrum for $\vartheta_{\Omega B} \sim 1$, $\vartheta_K^{\min} = 0$ and $\beta > 2$.

bunches $\dot{N}_B$ from a pulsar should be used, rather than e , m and $\dot{N}$. The quantities are related as $Q = |n_+ - n_-|e = \zeta ne$, $M = nm$ and $\dot{N}_B = \dot{N}/n$, where n_+ and n_- are the number of positrons and electrons in a bunch, respectively; $n = n_+ + n_-$, $\zeta = |n_+ - n_-|/n$ is the degree of charge separation in the bunches of ejected plasma. Substituting Q , M and $\dot{N}_B$ in equations (5) and (6) shows clearly that ω_m and ω_B do not depend on n ; there is, however, a relatively weak dependence of ω_m and ω_B on ζ ($\omega_m, \omega_B \propto \zeta^{2/(2+\beta)}$). If the number of particles n in a bunch is changing, the radiation spectrum (see Fig. 1) shifts along the $F(\omega)$ axis ($F(\omega) \propto n$ at $\zeta = \text{constant}$), although it does not change its shape.

Table 1 summarizes the α values of the curvature radiation flux as a function of frequency dependence ($F(\omega) \propto \omega^{-\alpha}$) for different frequency ranges, with β equal to 3, 4 or 5; ω_m and ω_B frequency values are also given for most probable parameters of pulsars^{8-10,12}: $\dot{N} = 10^{33}$ particles $\times s^{-1}$, $R = 1.4 \times 10^6$ cm, $\Gamma_0 = 800$, $\hat{\vartheta}_0 = 10^{-2}$ and with ζ assumed equal to 0.1. In this case, whatever, the value of β , the radiation spectrum extends to the frequency $(9/8)(c/R)\Gamma_0^3\hat{\vartheta}_0$, which is $\sim 1.7 \times 10^{11}$ Hz.

The following are the main observational data about low-frequency cutoffs in the pulsar radio emission spectra (for details see ref. 7). (1) The mean frequency of the pulsar radio emission maximum below which the radio emission flux decreases ($\alpha < 0$), is $\omega_m = 130 \pm 80$ MHz. (2) The mean value of the radio emission spectral index in the frequency range below the maximum ($\omega < \omega_m$) is -1.4 ± 0.4 . (3) The spectral indices of pulsar radio emission at frequencies higher or lower than the maximum frequency are almost equal in absolute magnitude and opposite in sign, that is, the steeper the radio emission spectrum up to its maximum ($\omega < \omega_m$), the steeper it is after it ($\omega > \omega_m$).

Table 1 shows that when $\beta = 4 \pm 1$ there is a good agreement between the expected behaviour of the curvature radiation spectrum, near its maximum, and the observational data. The ω_m versus β dependence is rather weak.

Above we have considered the case $\vartheta_K^{\min} = 0$, when in the pulsar rotation process its magnetic axis may coincide with the direction towards the observer. With the results from ref. 11 it is easily shown that the ω_m frequency value and the curvature radiation spectrum behaviour near the maximum at $\vartheta_K^{\min} \neq 0$ do not differ essentially from the case of $\vartheta_K^{\min} = 0$ if

$$\vartheta_K^{\min} < \vartheta_r \equiv \frac{8}{9} \frac{R}{c} \frac{\omega_m}{\Gamma_0^3} \hat{\vartheta}_0^2 \quad (7)$$

At $\omega_m = 130$ MHz, $R = 1.4 \times 10^6$ cm, $\Gamma_0 = 800$ and $\hat{\vartheta}_0 = 10^{-2}$, we have $\vartheta_r \approx 0.1$, that is, the ϑ_r value is sufficiently large ($\vartheta_r \gg \hat{\vartheta}_0$); hence for most pulsars $\vartheta_K^{\min} = 0$ may be assumed when the curvature radiation spectrum is considered to be near its maximum.

Note that the model presented here for the generation of pulsar radio emission due to the curvature mechanism may also

explain the radio emission spectrum behaviour at higher frequencies (see Fig. 1). Thus, the ω_B frequency (several GHz, see Table 1) falls into the interval of the observed values of the higher-frequency break of the pulsar radio emission spectrum^{1,7}. The observed values of the spectral indices after the high-frequency break are ~ 2 to 3 (refs 1, 7, 8), which corresponds to $(\beta - 2)$ for the same values, $\beta = 4-5$.

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Structure, stability and evolution of Saturn's rings

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Recent data obtained from the Voyager spacecrafts and ground-based measurements indicate: (1) the rings have a thickness of at most 150 m (ref. 1) and probably several times less^{2,3}; (2) the rings are mostly composed of ice particles ranging from centimetres to metres in size⁴; (3) the rings are subdivided into a large number of ringlets with a radial dimension ranging from 10-km down to the several metres resolution of the Voyager spacecraft's camera⁵; (4) the B ring contains very many optical depth variations (0.6-3)³. This behaviour is essentially determined by the collisional properties of the rings' ice particles. Here we report some preliminary results from an experiment designed to measure the coefficient of restitution of ice particles colliding at impact velocities relevant to Saturn's rings. We apply these results to simple dynamical models for Saturn's rings and deduce the rings' thickness to be < 5 m. We also show that regions with optical depth < 0.5 , such as the B ring, are unstable to viscous diffusion. Such an instability may be the cause of optical depth variations in the B ring.

The global properties such as the ring thickness and dynamical evolution time scale are basically determined by the collisional dynamics between the ring constituents and the resultant transport processes⁶⁻⁹. The cumulative effect of many collisions over a differentially rotating disk, such as Saturn's rings, is to establish a viscous stress which induces an outward transfer of angular momentum, an inward diffusion of mass¹⁰, and an energy transfer from systematic shear into dispersive motion at a rate $\dot{E}_t \approx v \Sigma \Omega^2 r^2$, where Ω and Σ are respectively the angular frequency and surface mass density of the disk at radius r . The kinematic viscosity of the disk, is defined by $v \approx \sigma^2 / [\Omega(\tau + \tau^{-1})]$ where $\tau = \Sigma A / m$ is the optical depth, A is the cross-section, m the mass of a typical ring particle, and σ is the average amplitude of the velocity dispersion. The particle collisions are partially inelastic and dissipate the kinetic energy associated with dispersive motion at a rate $\dot{E}_d \approx (1 - \varepsilon^2) r^2 \omega_c \Sigma \sigma^2$ where ε is the coefficient of restitution. The collision frequency, ω_c , is related to Ω such that $\omega_c = \tau \Omega$. A local energy equilibrium is established when $\dot{E}_t = \dot{E}_d$, that is,

$$(1 - \varepsilon^2)(1 + \tau^2) \approx b \quad (1)$$

where b is of the order of unity. Assuming that the rings are

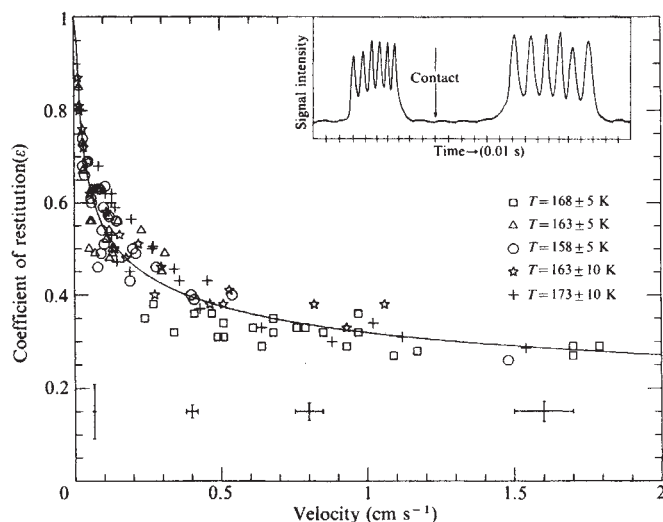


Fig. 1 A plot of the coefficient of restitution as a function of impact velocity for measurements at several different temperatures over the range 158–173 K. Estimates of the typical error bars are shown for four velocities at the bottom. The inset shows two sets of pulses obtained immediately before and after a collision. The values of ϵ and the velocity are obtained from the spacings of these pulses.

made of non-rotating, indestructable, and identical size ice spheres with a gaussian phase space distribution, b is determined from the rigorous treatment of the collisional Boltzmann equation⁸. An approximate analytical fit of these results indicates $b \approx 0.61$.

For most solid substances, ϵ is a function of the impact velocity, V (ref. 11). If an $\epsilon-V$ (or equivalently $\epsilon-\sigma$) relationship is available, it can be applied to equation (1) to deduce a $\tau-\sigma$ relationship. If a disk is made up of nearly equally sized particles, its r.m.s. thickness, H , would be $\sim 2f\sigma/\Omega$. Here f is introduced to account for the anisotropy of the velocity dispersion ellipsoid and is of the order of unity ($f \rightarrow 1/\sqrt{6}$ as $\tau \rightarrow \infty$)^{8,12}. Thus, an accurately determined $\epsilon-V$ relationship can be used to deduce H directly.

Another reason for determining an $\epsilon-V$ relation is associated with the viscous stability of disk flow. Several authors^{13–15} have suggested that the condition for thermal and viscous stability cannot be satisfied simultaneously if $\tau \gg 1$. In these conditions the rings have a tendency to break up into many ringlets. This mechanism seems to offer a viable explanation for the origin of the density variations in the optically thick B ring where tidal effects due to various saturnian satellites are negligibly small. Physically, if the viscous couple, $W = 3\pi\Omega r^2 \Sigma v$, increases with τ , the rings are stable whereas if W decreases with τ the rings are unstable. The $W-\tau$ relationship can also be derived from an $\epsilon-V$ relationship. We have designed an experiment and determined this $\epsilon-V$ relationship in a laboratory environment.

To measure ϵ for ice particles in the conditions assumed to exist in Saturn's rings, the apparatus must meet several criteria. First, the relevant impact velocity must be in the range 0.01–5 cm s⁻¹. Second, the frictional damping of the system must be small compared with the kinetic energy associated with ice particle collisions such that the damping correction factor is relatively small. Third, the relevant ambient temperature should be in the range 100–150 K. Fourth, before and after each collision, the ice particles must be separated by a sufficiently large distance, say >0.01 cm, such that tiny powdered ice chips on their surface do not induce strong interactions between them except during the course of each collision. Finally the collisions should resemble free collisions as closely as possible.

The simplest experimental system which can satisfy most of the above requirements is to attach an ice ball to an oscillator and let it strike a stationary block of ice. A sufficiently low impact velocity and a reasonably large oscillator amplitude can be achieved with a compound pendulum with the axis of rotation

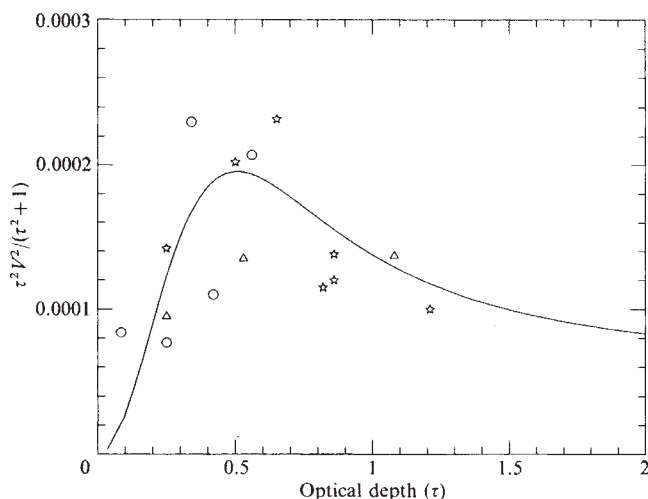


Fig. 2 A plot of $\tau^2 V^2 / (\tau^2 + 1)$ as a function of optical depth τ . The values for τ were obtained using equation (1). The points are obtained from the data in Fig. 1 and the solid line is a graph of $\tau^2 V^2 / (\tau^2 + 1)$ versus τ according to equation (4).

very close to the centre of mass. In this case, the angular frequency is $(Mgd/I)^{1/2}$ where M and I are the mass and moment of inertia of the system. We constructed such a system using a disk mounted on an aluminium rod. Small weights at the top and bottom of the disk allow us to adjust the distance between the centre of mass and the suspension (that is rotation) axis, d . An ice ball is attached to one edge of the disk and a counter weight on the other edge in a manner such that their centre of mass is at the centre of the disk. The entire system is suspended by knife blades resting on polished agate plates. With this system, we have achieved periods as long as 60 s with the Q -value equal to 4. For shorter oscillation periods, such as $T < 30$ s, $10 < Q < 30$.

The main limitation of the above system is that collisions are not entirely free. The coupling between the ice particles and the pendulum body will cause a slight elastic deformation in the pendulum during a collision. However, we note that most of the kinetic energy dissipation occurs as a result of fracturing at the tiny contact surface area of the ice particles. For typical impact velocities the time interval during contact is considerably longer than the sound crossing time across the ice balls. Our experiments indicate that a sufficiently rigid mounting of the ice particle onto the pendulum effectively eliminated temporary redistribution of elastic energy within the system and thereby provided a good measure of ϵ .

The ambient temperature of the system is set at 150–175 K using a simple cryostat constructed from styrofoam and cooled with liquid nitrogen. The ice-block temperature is measured with an Omega thermocouple thermometer frozen into the ice block. The velocity of the ice ball is measured by reflecting a laser beam off a mirror mounted on the rotation axis of the pendulum. The reflected beam passes over a photocell masked with five slits 1 mm wide, 2 mm apart and situated 4 m from the pendulum. The photocell is positioned close to the point which corresponds to contact. Five pulses produced immediately before and after each collision provide an accurate measurement of the incoming and outgoing velocities from which ϵ can be deduced. Five slits are used primarily to improve the accuracy of the measurement and to eliminate damping contributions associated with the finite Q value of the pendulum. The results of our experiments indicate that only a small, that is $<2\%$, correction is ever needed to account for damping.

A typical pulse train and the summary of the results obtained from the four lowest temperature runs are illustrated in Fig. 1. The general behaviour of ϵ as a function V is typical of the data taken at other temperature ranges. The data can be qualitatively divided into three cases. At relatively large V , ϵ changes little over a wide range of impact velocity. Below $V \approx 0.25$ cm s⁻¹,

ϵ increases rapidly with decreasing V . The extrapolation of our data indicates that, at a sufficiently small but finite velocity, ϵ approaches unity. This behaviour at the low velocity limit is consistent with the classical theory of nearly elastic deformation associated with low-velocity impacts¹⁶⁻¹⁸. However, the asymptotic plateau for relatively large velocity cannot be accounted for by any existing collision model.

The data presented in Fig. 1 can be fitted remarkably well with a power law

$$\epsilon = (0.32 \pm 0.02) V^{-0.234 \pm 0.008} \quad (2)$$

where V is the impact velocity measured in cm s^{-1} for the range $0.015 < V < 5.1$. Extrapolation of this power law indicates that $\epsilon \rightarrow 1$ for $V < V_c = 0.008 \text{ cm s}^{-1}$. The dispersion of the data, relative to the best fitted curve, is much larger for intermediate collision velocities than for large velocities, where ϵ attains an asymptotic value of 0.25, and for very small velocities where ϵ approaches unity. Special care has been taken to eliminate various possible external causes for this large dispersion. A trial experiment for collision between a solid synthetic rubber ball (super ball) and a rigid surface yielded an ϵ - V relationship with dispersion of order less than one-tenth those found for ice ball-block collisions. Clearly, the scattering of the data is considerably larger than the typical measurement uncertainty which is indicated in Fig. 1. We propose that the large dispersion in the data is real, and is probably associated with compression and random powdering of tiny ice chips on the surface. Irregularities in the shape of the ice chips can lead to considerable variation in the radius of curvature and geometry of the contacting surfaces of the ice ball and block. The tendency for the dispersion to decrease at relatively large impact velocities is probably caused by the surface distortion during a collision being large enough to override any variation in the surface geometry immediately before each collision.

Note that the effective mass involved in a collision is 5.4 times larger than the mass of the ice ball mounted to the pendulum. Because the ice block is held stationary, it has an infinite inertial mass. In any event, as Saturn's rings are composed of ice particles with a large range in size, it is desirable to investigate collisions between particles with various masses and sizes. These investigations are underway and the results will be presented elsewhere.

Despite recent considerable observational and theoretical advances in our understanding of Saturn's rings, the actual physical nature of the constituent particles remains unclear. The rings may either be made of solid particles with relatively smooth surface radius of curvature or particles covered with loose ice chips¹⁹. The amount of gas and dust inclusions in the ring particles is also unknown. In our experiments, both the ice ball and ice block are prepared with a smooth surface and are relatively free of gas or dust. (The amount of surface frost on the ice ball has also been minimized although this can never be eliminated.) Furthermore our data are obtained for direct collisions whereas grazing collisions may be far more common in Saturn's rings. Thus, it is not entirely clear to what extent the data obtained from the above experiment are relevant to the structure of Saturn's rings. Nonetheless, we apply our results to the standard uniform-size hard sphere model⁸ for simplicity. Under this assumption, V_c defines a lower limit on the thickness of the disk which is $\sim V_c/\Omega = 50(V_c/0.01 \text{ cm s}^{-1})(10^{10} \text{ cm}/r)^{3/2} \text{ cm}$. If we apply the results of equation (2) to equation (1), it follows that

$$H = 2f\sigma/\Omega = 80f[1 - b/(1 + \tau^2)]^{-2.1}(r/10^{10} \text{ cm})^{3/2} \text{ cm} \quad (3)$$

Applying an idealized, uniform-particle sized model, we derive for most regions of the ring that $H < 5 \text{ m}$, provided the particle size is smaller than H . This value is consistent with that deduced from microwave scattering experiments². The slight difference between these values and those deduced from resonant damping analyses³ may be due to the additional energy source associated with external perturbers.

In the absence of external stimulation, such as Saturn's satellite resonances, the energy equilibrium implies an effective viscosity

$$Nu \approx 0.3(r/10^{10} \text{ cm})^{3/2} \frac{[1 - b/(1 + \tau^2)]^{-4.2}}{(\tau + \tau^{-1})} \text{ cm}^2 \text{ s}^{-1} \quad (4)$$

and a corresponding diffusion time $T_d \approx r^2/\nu$, longer than the age of the Solar System. The corresponding viscous stress is plotted in Fig. 2. Superimposed on the actual data is a W - τ relationship derived from equation (2) such that

$$W \sim [1 - b/(1 + \tau^2)]^{-4.2} \tau^2/(1 + \tau^2) \quad (5)$$

The maximum value of W is attained at $\tau_c = (1 - b)/(4.2b - 1)$. The scatter of the original data (Fig. 1) is amplified in Fig. 2 by numerous mathematical transformations of the experimental data. The results in equation (5) imply that, for $\tau \geq 0.5$, W decreases with τ and therefore the rings are unstable. For $\tau \leq 0.5$, the rings are stable. If we were to take the widely scattered data points, the critical τ would lie in the range 0.3–0.7. Thus, the optically thick regions of the disk are expected to be diffusively unstable and would open up low density regions with τ of the order 0.5–0.7. The observed optical depth profile of the B ring indicates that the typical τ in the less opaque regions of the B ring is ~ 0.6 (ref. 3).

In the above discussion we have not considered the effect of particle size distribution. Note that the large, metre-size particles have radii comparable to or larger than the ring thickness, as derived from equation (3), such that they may form a 'monolayer'. For these particles the velocity dispersion is determined by the gradient of the rings' keplerian velocity across their diameter⁷ so that their W monotonically increases with τ and their motion is viscously stable. Consequently, the small centimetre-size particles and the large metre-size particles in the high τ B ring may have rather different radial structure. Voyager's data indicate that the primary contribution to the τ variation in the B ring is from the small particles. The large particles have a relatively smooth radial distribution. Thus, a realistic model for the evolution of the B ring requires a detailed analysis of the collisionally induced transport process for a collection of particles with different sizes (G. Stewart, in preparation). We intend to supplement the experimental data presented here with data on collisions between ice balls with different radii of curvature at the contact point.

The above results may also be applied to study the actual damping process in resonant excited density waves^{20,21}, the structure of narrow rings and sharp edges as well as the satellite shepherding phenomenon²². As for our experiments, variations in ice ball's and ice block's surface geometry, surface crystal lattice structure, collisional grazing angles, and ambient temperature will be examined in future. In general, we believe that the results presented here are a first step towards understanding ice-particle collisional processes.

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Topographic EXAFS

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The EXAFS (or extended X-ray absorption fine structure) method of absorption-edge spectroscopy for the determination of crystal bond lengths and coordination number, especially in the first coordination shell, is in very widespread use in chemical, crystallographic, biological and to some extent metallurgical research. The high-resolution method of X-ray diffraction topography, which reveals crystal defects, is likewise widely used in studies on crystal growth, phase transformations, chemical decomposition and mechanical properties and domain studies: that is, in the area of 'microstructure' rather than 'crystal structure'. It is obvious that the techniques are complementary and that both could be required to solve a particular problem—structural changes induced by segregation at a surface or internal interface, for example. However, up to now, EXAFS data have been taken only as an average over large samples, thus losing all microstructural information, and topographic data have not provided structural, bonding or chemical information. We present here the first experimental method of combining these experiments, simultaneously on one specimen.

The method relies on the properties of synchrotron radiation (SR)¹, whose high intensity and continuous spectrum enables EXAFS spectra to be accumulated in reasonable times (some tens of minutes) and X-ray topographs to be taken in some seconds. An obvious way of combining the methods would be to take an X-ray topograph to locate areas of interest, place an aperture of diameter equal to the resolution required in this area and measure successive EXAFS spectra during a linear or raster scan of the area. This method would certainly provide the best spectral data, but a rapid calculation of available intensities shows that with most current SR sources the data collection times would be quite impractical—days or weeks.

A possible way of speeding up the spectral data collection is the use of white radiation incident on the sample, followed by a dispersing element (for example, bent crystal) and a film or linear detector; this has recently been achieved^{2,3} and results in dramatic decreases in data collection times. The topographic data can then be collected in a separate exposure.

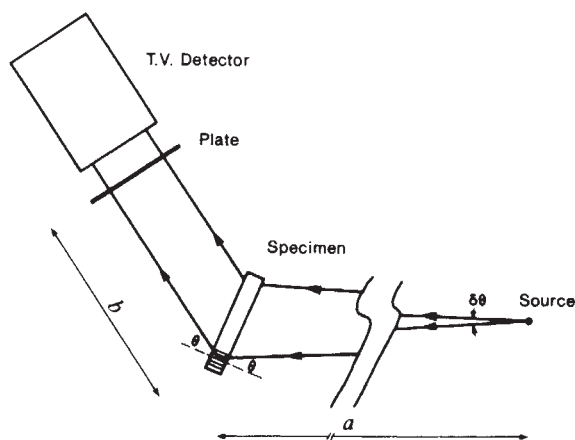


Fig. 1 Experimental arrangement for white beam topography. Typical specimen–source distances, *a*, are 20–80 m and specimen–film distances, *b*, 10–100 mm.

If a good quality single crystal is being used as the specimen, as it often is when topographic information is sought, a further and very simple technique is to use the specimen as its own monochromator. In the white radiation topographic method (Fig. 1), the incident beam diverges from the SR source, which is some tens of metres distant from the specimen. There is therefore a small variation of incident Bragg angle across a specimen, and a consequent variation of wavelength (energy) selected by the specimen for diffraction.

The experimental procedure is as follows. The orientation of the specimen is chosen and set so that the wavelength selected by some part of the specimen is at the absorption edge under

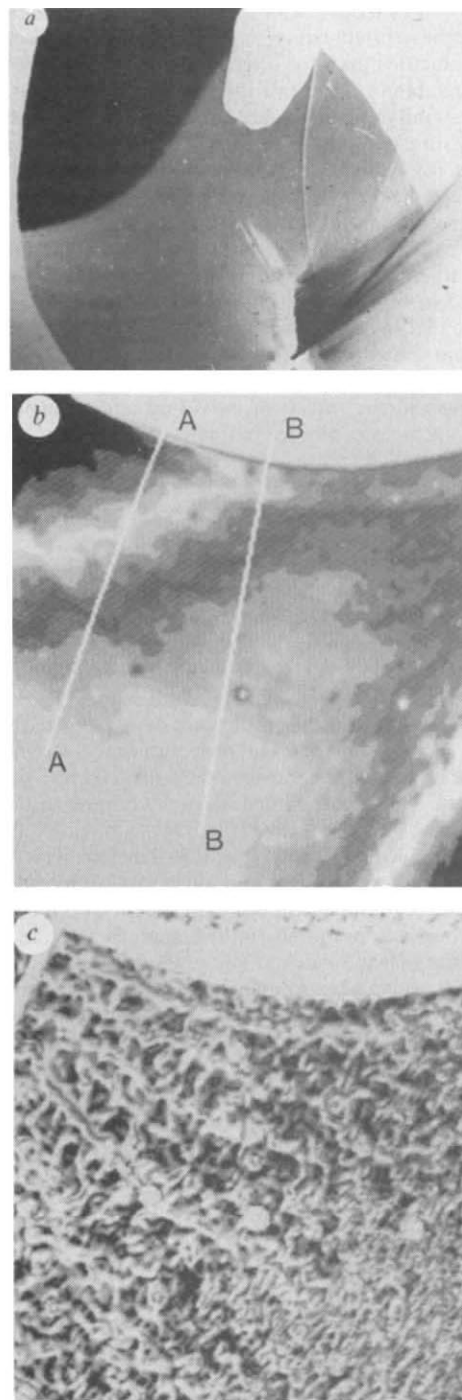


Fig. 2 *a*, White radiation topograph of Nb specimen (near perfect but with some elastic bending). 002 reflection, $\lambda = 0.65298 \text{ \AA}$ at the absorption edge. SRS parameters 2 GeV 86 mA, 25 s exposure on Ilford L4 plate. *b*, Digitized and image processed (smoothed, threshold cut and contrast stretch) version of *a*. Image are $2.24 \times 2.24 \text{ mm}$. *c*, Differentiated version of *b*.

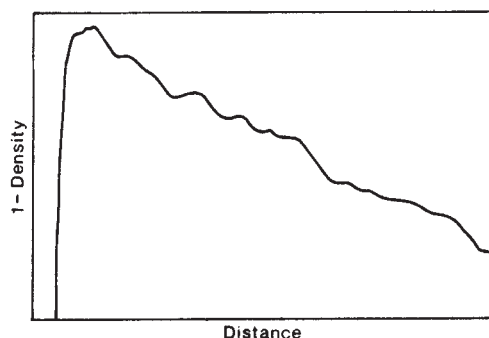


Fig. 3 Density traces extracted from Fig. 2, along line AA.

study. A standard topographic image is then recorded on high resolution plate, for example Ilford nuclear emulsion L4, exposed so that the high absorption side of the image is in good contrast. The wavelength selected by a point on the image is a direct function of the distance on the image from the edge, measured normally to the latter. The diffracted beam falling on each point will therefore have suffered scattering and absorption characteristic of a given photon energy. This results in 'fringes' in the image, parallel to the image of the edge, corresponding to maxima and minima in the EXAFS spectrum.

The first results, taken on the white radiation camera⁴ at the SRS, Daresbury Laboratory, are shown in Fig. 2. Figure 2a shows the standard topographic image of a 125- μm thick Nb specimen at the Nb $K\alpha$ edge, reproduced photographically. The absorption edge is clear, but the fringes have weak contrast and are not evident. However, image processing techniques can be used to reveal them. The original plate was digitized on a Joyce Loebel flat bed densitometer with 5- μm resolution and the resulting matrix digitally smoothed and processed to cut off low and high values and to stretch the contrast. The result is shown in Fig. 2b; Fig. 2c shows a differentiated version of Fig. 2b. In each of these, especially the latter, the fringes are easily visible. Figure 2 shows density variations along the line marked AA in Fig. 2b. These show, albeit noisily, a typical EXAFS spectrum.

To demonstrate that these are indeed EXAFS-type data, comparison was made with an EXAFS spectrum taken on a rotating-anode generator of a 25- μm thick Nb foil. A rotating anode generator with a Mo target and a Si 800 bent crystal mono-

Table 1 Comparison of X-ray energy at spectral minima in data extracted from the topograph (Fig. 2b) and from a standard EXAFS spectrum

Fringe edge	From topograph (keV)	From spectrum (keV)
	18.99	18.990
1	19.07	19.074
2	19.13	19.124
3	19.19	19.180
4	19.24	19.252
5	19.29	19.330

Estimated errors are ± 0.01 keV from the topographic data and ± 0.005 keV from the spectral data with the exception of fringe 5 (rather broad) where they are each about ± 0.02 . The topographic data were calibrated relative to the edge taken as 18.99 keV.

chromator were used. The spectrum in Fig. 3 was calibrated for energy by comparison with the absorption edge positions in a set of topographs in which the specimen was rotated by 0.025° steps between each topograph. A composite photograph of the successive topographs is shown in Fig. 4. This provides an internal calibration that eliminates uncertainties due to curvature of the specimen, though it would not be necessary on a truly flat specimen. The energies corresponding to minima in the EXAFS spectrum are shown in Table 1 for the present results and the standard spectrum. The agreement leaves no doubt that this white-beam method measures true EXAFS data, simultaneously with the topographic image, with an exposure time of 25 s in this case.

We need to know what is the quality of the EXAFS spectra obtained by this means (the images show normal topographic resolution and contrast). There are several factors to be considered: (1) dispersion and energy range; (2) effect of finite source height; (3) effect of source divergence; (4) wavelength acceptance of crystal reflection; (5) spatial resolution of photographic plate. Let the source height be h , the source-specimen distance a , the specimen-detector distance b , and the Bragg angle θ . ψ_0 and ψ_h are as usual the angles made by the incident and diffracted beams with the inward-facing surface normals and $\gamma_0 = \cos \psi_0$, $\gamma_h = \cos \psi_h$. Examples of the application of the equations will be given for the case corresponding to the

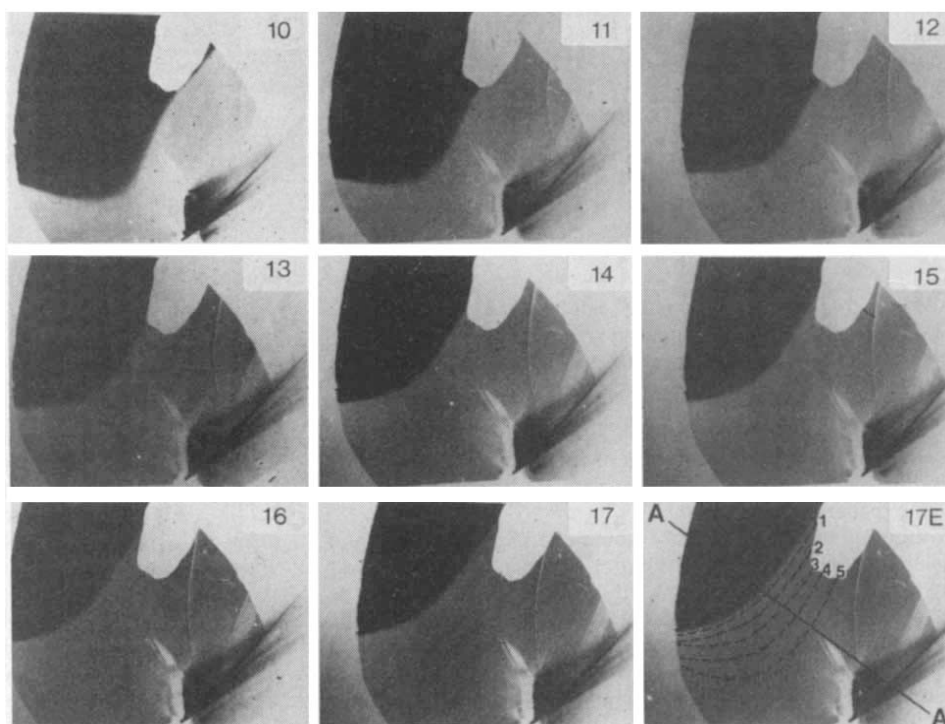


Fig. 4 Composite topograph of area shown in Fig. 2, showing successive positions of absorption edge as specimen rotated in 0.025° steps.

experiment reported above: Nb 002 reflection, $\gamma_0 = \gamma_h = 1$, d -spacing 1.6503 Å, $\theta = 11.4105^\circ$ at the K absorption edge which is at 0.65298 Å, $a = 81$ m, $b = 75$ mm.

The position of a point on the image is defined by its perpendicular distance x to the absorption edge, with the photographic plate set normal to the diffracted beam. Straightforward geometry gives, assuming for the moment a point source, a dispersion relation:

$$\frac{\delta\lambda}{\lambda} = \frac{\gamma_0}{\gamma_h} \cot \theta \frac{x}{a} \quad (1)$$

if b is small, otherwise (because the diffracted rays converge from the specimen)

$$\frac{\delta\lambda}{\lambda} = \cot \theta \frac{x}{a \frac{\gamma_h}{\gamma_0} - b} \quad (2)$$

For the Nb example this gives a wavelength or energy dispersion of 3.06×10^{-7} per 5 μm digitizing element. Over a 10-mm image, the range is thus 6.12×10^{-4} . These figures correspond to 0.0058 eV resolution and 11.6 eV range. This spectral resolution cannot be achieved in practice for various reasons, discussed below, but the range in the Nb sample discussed above was ~ 300 eV over 2 mm (from internal calibration), therefore the specimen was curved with ~ 0.63 m radius of curvature. One purpose of recording the absorption edge position as a function of inclination was to determine this curvature, near a notch in the specimen. The dispersion range is thus adequate for XANES (X-ray absorption near-edge structure) measurements on a flat specimen, but for a full EXAFS spectrum several topographs should be taken at different inclinations. Most SR beam lines are much shorter than 80 m and so greater energy spread would occur.

The finite source height (in the incidence plane) has a great effect on the resolution. Imagine a parallel beam the height of the source falling on the specimen. This will be diffracted to a beam the same height (multiplied by the asymmetry factor γ_h/γ_0) and be recorded on the film, blurring spectral data in this region. The minimum useful area on the film is therefore $h\gamma_h/\gamma_0 = x_{\min}$. This can be combined with the dispersion equation (1) to give the minimum spectral resolution

$$\frac{\delta\lambda}{\lambda_{\min}} = \frac{h \cot \theta}{a} \quad (3)$$

(with a corresponding equation if equation (2) is used). For the niobium example with the SRS FWHM value of $h = 300 \mu\text{m}$, we obtain for the Nb example

$$\frac{\delta\lambda}{\lambda_{\min}} = \frac{\delta E}{E_{\min}} = 1.84 \times 10^{-5} \text{ or } 0.35 \text{ eV}$$

If now x is taken to be $h \gamma_0/\gamma_0$ then the apparent source divergence is $2h/a$. For the SRS at $a = 81$ m, this results in

$$\frac{\delta\lambda}{\lambda} = \cot \theta \ 7.4 \times 10^{-6} \quad (4)$$

For Nb₀₀₂, this becomes 3.7×10^{-5} , ~ 0.7 eV.

The effect of the wavelength acceptance of the crystal is found from the FWHM of the rocking curve as $\delta\theta$, using $\delta\lambda = \cot \theta \delta\theta$. For an appropriate FWHM ~ 5 arcs, this gives

$$\delta\lambda = 1.72 \times 10^{-4} \text{ or } 3.25 \text{ eV}$$

The effect of the spatial resolution of the plate $\leq 1 \mu\text{m}$, is quite negligible on the spectral resolution ($\delta\lambda/\lambda \sim 10^{-7}$), because the intrinsic dispersion is so high.

Combining these three effects (source height, divergence and wavelength spread) by taking the root of the sum of the squares leads to an energy resolution in the Nb case of 1.3×10^{-4} or ~ 3.4 eV. This is perfectly acceptable for the purpose. The dominant factor is the wavelength acceptance of the crystal, and this could easily be decreased by using a weaker reflection.

The signal-to-noise ratio on the spectrum in Fig. 3 is poor and not normally acceptable for a useful EXAFS spectrum:

note that the specimen was about five times thicker than the ideal. However, only 4 pixels were involved in each smoothing operation, giving 20 μm spatial resolution, whereas the minimum useful pixel size is $h\gamma_h/\gamma_0 = 300 \mu\text{m}$ in this case. Because nuclear emulsion can record up to about 5 photons μm^{-2} at this wavelength, smoothing over a 300- μm range would give a dynamic range of $5 \times 300^2 = 4.5 \times 10^5$ and a corresponding precision of 0.15%. Whilst this is still not as good as the best EXAFS experiments it is acceptable for many purposes.

What then are the applications of this technique? Whilst it is a rapid and convenient way of obtaining EXAFS spectra if good crystals are available, often they are not, at least without considerable effort. The interest must lie in the ability to perform chemical and defect crystallography on the same sample, as can be performed (on very different samples) by the electron energy loss method⁵. Applications such as structural changes near interfaces with segregation or at decorated dislocations, valence state changes in defect ionic compounds (for example, iron salts, some of which are important catalysts), structural changes at the onset of chemical decomposition are possible. Whilst in principle one could scan the specimen in orientation to reconstruct the complete spectrum for each pixel on the image this would probably not be necessary in practice. A structural or chemical change at a pixel, from sufficient volume of the specimen, would result in a distortion of either the position or the intensity of the local fringe pattern. This would often provide the clue to anomalous behaviour, which could be followed up with techniques such as microbeam EXAFS over a small, closely defined area, or microbeam trace element analysis, all using the same apparatus.

It is also apparent that similar contrast is to be expected in radiographs taken near absorption edges with non-dispersive monochromators. With brighter and more intense sources, TV detectors and image processors, dynamic topographic EXAFS should be a real possibility.

We thank Professor A. J. Forty (University of Warwick) who first encouraged us to look for techniques of microbeam EXAFS, and Dr P. Georgopoulos (Northwestern University) for help in obtaining the reference Nb EXAFS spectrum, and Daresbury Laboratory staff for cooperation and the SRS facilities. The work was carried out with the support of the UK SERC (grant GR/B61283 and the US ONR (contract N00014-C-1012).

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Natural chlorine isotope variations

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Previously known natural variations in the stable isotope ratio of chlorine, $^{37}\text{Cl}/^{35}\text{Cl}$, have been within the experimental error of $\pm 1.0\%$. [All our measurements are reported in terms of δ^{37} in units of ‰; $\delta^{37}\text{Cl} = [(R_x/R_s) - 1] \times 1,000$, where $R = ^{37}\text{Cl}/^{35}\text{Cl}$.] Using methyl chloride gas in a ratio mass spectrometer, we have increased the precision of measurement to $\pm 0.24\%$, and report here significant isotope variation in natural chlorides. In general, salt deposits and saline hydrothermal springs tend to be enriched in ^{37}Cl with respect to seawater. Groundwater shows both enrichment and depletion with respect to seawater. Saline groundwater becomes more enriched in ^{37}Cl with increasing depth.

Eighty years of chlorine isotope studies¹⁻⁹ have shown that the stable isotope ratio, $^{37}\text{Cl}/^{35}\text{Cl}$, is remarkably constant in nature. Hoering and Parker⁷ used HCl gas in a mass spectrometer to measure stable chlorine isotope ratios in 81 samples of natural materials; they found no significant variation beyond their stated limit of 1.0%, although they did report two samples, both from a deep aquifer brine, as being possibly fractionated. Rankama¹⁰ summarized the bulk of chlorine isotope studies when he wrote, "[Past work] suggests that additional accurate measurements of the isotopic constitution of chlorine in carefully selected samples are required to investigate the possible natural fractionation of the chlorine isotopes". We have used a technique of measurement which is more precise than those used in previous studies, and focused on comparing subsurface brines with seawater and other natural chlorides.

The measurements reported here were made by mass spectrometry of methyl chloride ions (CH_3Cl^+), using the method of Taylor and Grimsrud¹¹ to prepare methyl chloride gas. Chlorine is quantitatively precipitated as silver chloride (AgCl) from aqueous solutions with silver nitrate. AgCl is then reacted with methyl iodide for 2 days in a reaction vessel at 70–100 °C. The product, CH_3Cl , is then separated from excess methyl iodide by gas chromatography and yields measured to ensure that all of the chlorine is recovered. An isotope ratio mass spectrometer (V. G. Micromass 602C) compared sample CH_3Cl with a commercially-produced reference gas to a precision of $\pm 0.03\%$. The total analytical standard deviation, determined from more than 20 replicates of our laboratory standard NaCl over a 1-yr period, is $\pm 0.24\%$. Following Taylor and Grimsrud's example, this standard deviation of 0.24% is used as the precision for the measurement technique. The mass spectrometer collector slit width masks all but the $m/e = 52$ peak for the minor beam and the $m/e = 50 + 51$ for the major beam. Variations of $^{13}\text{C}/^{12}\text{C}$ and D/H are minimized by using the same batch of methyl iodide and repeated standardization. Fragmentation patterns should be the same for equally pure sample and reference methyl chloride on a given day's run, and in practice seem not to vary significantly even with filament changes. Frequent monitoring of the constancy of the 51/(49 + 50) difference between reference and sample ensures the validity of the data.

Repeated measurements of eight oceanic chloride samples, one from the Gulf of Mexico off the coast of Texas and the others from depths up to 600 m in the Pacific Ocean, gave a total spread of 0.12% and a standard deviation of 0.05%. Results reported here are relative to this standard mean oceanic chloride

(SMOC) isotope ratio ($^{37}\text{Cl}/^{35}\text{Cl}_{\text{oceans}} \approx 0.324$), thus the average $\delta^{37}\text{Cl}$ for ocean water is set at 0.00%.

Table 1 shows the results of several groups of samples that we have analysed. Chlorine isotope ratios of aquifer brines from east Texas and Louisiana, including those shown in Table 1, are plotted in Fig. 1 as a function of approximate depth of sample collection.

Table 1 indicates that significant chlorine variation in natural environments does occur, although most of the variation is within 1.0% of ocean water chlorine. The range of variation shown is $\sim 2.1\%$ with a precision of 0.24%. Although little can be said about the variations in terms of mechanisms of fractionation, the measurements indicate some distinct geochemical tendencies. Salt deposits tend to be enriched in ^{37}Cl with respect to seawater, as do hydrothermal samples. Groundwater samples may be either enriched or depleted in ^{37}Cl with respect to seawater. The two shallow groundwater samples shown in Table 1 are from the same aquifer, suggesting that within one aquifer there may be more than one chloride source and/or isotope fractionation mechanism. In brines from deep aquifers, increasing isotope enrichment seems to correlate with increasing depth (Fig. 1). Total chloride concentration of these brines also correlates with isotope composition within each aquifer.

To date, all of the samples measured in our study contain chlorine as chloride ions which limits the likely mechanisms for enriching isotopes to precipitation and diffusion processes. Na^{37}Cl may precipitate more readily than Na^{35}Cl just as $^{34}\text{SO}_4$ precipitates more readily than $^{32}\text{SO}_4$ during gypsum formation¹². Measurements on NaCl deposits in Table 1 may reflect isotope fractionation during formation. Counter-current electromigration, a laboratory method used to separate chlorine isotopes¹³, may be analogous to chlorine isotope fractionation in groundwater (F. Phillips and H. B., in preparation). During counter-current electromigration experiments, chloride in a NaCl solution is driven through a sand-packed tube by an electrostatic force against the convective movement of the solution. ^{35}Cl

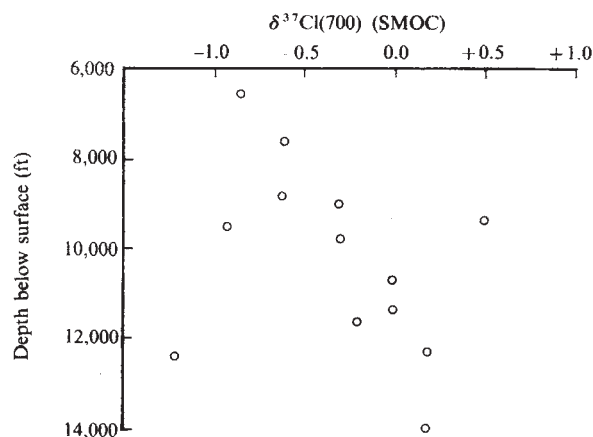


Fig. 1 Variation of $\delta^{37}\text{Cl}$ with depth of sample collection in oil field brines from east Texas and Louisiana. The Pearson product moment correlation coefficient for these data is +0.61 and is significant to within the 95% confidence limit^{14,15}. Excursions from the apparent linear trend may represent vertical mixing of brines through geological heterogeneities and cracks in well casings. The correlation of ^{37}Cl enrichment with depth may reflect an isotope fractionation mechanism related to the electrostatic properties of subsurface clays (ref. 13 and F. Phillips and H.B., in preparation).

Table 1 Results of analyses

	$\delta^{37}\text{Cl}$ (‰)*	Student's <i>t</i> -test results (probability of variance from SMOC ^{14,15})
Ocean water samples		
(14 measurements on eight samples, s.d. 0.05%)	0.00	—
Halite samples		
Bedded Salt, Salina Formation, Ohio (mean of 21 measurements, s.d. 0.24%)	+0.52	>0.99
Weeks Island Salt Dome, Louisiana (mean of four measurements with total spread of 0.08%)	+0.20	0.98
Hydrothermal samples		
Morgan Hot Spring, California	+0.40	0.92
Crater Lake, New Zealand	+0.45	0.98
El Chichon crater water, Mexico	+0.41	0.95
Shallow groundwater samples (<150 m)		
Milk River aquifer sample no. 17, Canada	+0.8	0.98
Milk River aquifer sample no. 1, Canada	-0.4	0.96
Deep groundwater samples (>2,000 m)		
WC-W155, Louisiana	-0.36	0.85
81-1085, Texas	-0.62	>0.99
82-090, Texas	-0.95	0.95
81-1082, Texas	+0.53	>0.99
81-1124, Texas	-1.29	>0.99

* All data reported with respect to SMOC.

diffuses more effectively than ^{37}Cl towards the cathode end of the tube. In a groundwater analogue of counter-current electromigration, the electrostatic force may be provided by charged clay mineral surfaces and streaming potential produced by forced convection of water. The convective movement is induced by natural hydraulic and osmotic pressure gradients. Although discussions of fractionation mechanisms based on our results are speculative, experiments and the construction of theoretical models designed to clarify the nature of possible mechanisms are underway.

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Suboxic diagenesis in banded iron formations

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Anomalous isotopic composition has been reported for the carbon in carbonate minerals of banded iron formations. Well studied examples show an enrichment in the light isotope of carbon, ^{12}C . This enrichment presumably reflects unusual circumstances in the deposition of these sedimentary rocks. It is suggested here that the isotopically-light carbonate results from early diagenetic oxidation by bacteria of substantial amounts of isotopically light organic carbon. The electron acceptor that permits oxidation in the absence of free oxygen is presumed to be iron(III) which may have been significantly more abundant in the initial chemical precipitate than in the post-diagenetic sedimentary rock.

Banded iron formations are chemical sediments, formed by precipitation of dissolved species from solution¹. They are composed principally of cryptocrystalline or crystalline quartz, iron oxides (haematite and magnetite), iron silicates (greenalite and related minerals) and iron carbonates (siderite and ankerite)². Banded iron formations are major sources of iron ore. Essentially all of them were deposited before $\sim 1.7 \times 10^9$ yr ago³, an observation that has been interpreted to reflect a transition at about this time from a hydrosphere devoid of oxygen to a hydrosphere rich in oxygen^{4,5}. Because oxidized iron(III) is almost insoluble in all but very acid solutions, the transport of iron in solution and its subsequent chemical precipitation in banded iron formations must have occurred in anoxic waters. The extensive area of some banded iron formations and substantial rates of accumulation of iron and silica seem to imply deposition in basins that were open to the ocean⁶⁻⁹.

The isotopic composition of the carbonate minerals in banded iron formations has been studied most thoroughly in formations

of the Hamersley Basin of Western Australia^{9,10}. These undeformed sedimentary rocks have been subjected to only low-grade metamorphism¹¹; they date from $\sim 2.5 \times 10^9$ yr ago¹² and appear to have been deposited on a submarine platform or bank protruding into or marginal to an ocean⁹. Carbonate minerals are fairly abundant, typically making up between one-tenth and one-half of the rock^{10,13}. Becker and Clayton¹⁴ studied the isotopic composition of the carbonate minerals in the Dales Gorge Member of the Brockman iron formation and reported an enrichment in the light isotope of carbon. Expressed as the departure of the ratio of carbon isotopes from the ratio in a standard carbonate, this departure amounted to an average of $\sim 10\%$, expressed as $\delta^{13}\text{C} = -10\%$. This finding has been confirmed and extended by Baur *et al.*¹⁰, who studied the underlying Marra Mamba and Mt Sylvia formations as well as the Dales Gorge Member of the Brockman iron formation. These authors found variable isotopic compositions, but in all cases the carbonate minerals were strongly enriched in the light isotope of carbon. Similar results for banded iron formations in North America have been reported^{15,16}.

Little isotopic fractionation with respect to dissolved carbonate results from the precipitation of carbonate minerals including the iron carbonates, siderite and ankerite¹⁷, so most marine carbonates have isotopic compositions close to the standard, $\delta^{13}\text{C} \sim 0\%$. Indeed, isotope measurements on marine carbonate rocks of different ages are used to infer the history of the carbon isotopic composition of seawater. Such studies have revealed only minor variation of seawater carbon from a value near $\delta^{13}\text{C} = 0\%$ since at least 3.5×10^9 yr ago¹⁸⁻²⁰. There is no reason to suppose that the isotopic composition of the ocean was any different at the time of deposition of the banded iron formations of the Hamersley Basin. Indeed, the Wittenoom Dolomite^{10,14}, a non-iron formation carbonate rock immediately overlying the Marra Mamba iron formation, has a carbon isotopic composition of $\delta^{13}\text{C} = 0.36\%$ very close to the marine carbonate standard. It does seem likely, therefore, that the carbonate minerals in the iron formations were deposited with isotopic compositions different from that of the seawater from which these minerals were ultimately derived.

Isotopically-light marine carbonate in Phanerozoic sedimentary rocks would generally be attributed to the oxidation of reduced organic carbon during early diagenesis²¹. Reduced organic carbon is strongly enriched in the light isotope, $\delta^{13}\text{C} = -25\%$ or lower^{22,23}. Diagenetic oxidation of organic carbon in young sediments can dilute the seawater carbonate dissolved in the pore waters of the sediments, yielding isotopically light dissolved carbonate²⁴. The carbonate minerals that equilibrate with these pore waters can also be isotopically light. Data on isotopically light marine carbonates of Mesozoic age, presumably produced in this way, have been presented by Irwin *et al.*²¹. Could this process not also have been responsible for the isotopically light carbonates in banded iron formations, a possibility suggested by Baur *et al.*¹⁰?

The problem with this mechanism is to identify the electron acceptor that made possible the oxidation of organic carbon in iron formations. The assemblage of minerals in these formations provides clear evidence for a very low oxygen fugacity in the pore waters of the sediment². This conclusion is not contradicted by the presence of some iron(III), which is stable even at low oxygen fugacities. So the electron acceptor was not oxygen. There is also no reason to suppose that dissolved nitrate was abundant on the anoxic Earth²⁵. But the oxidation process evidently did not involve the reduction of dissolved sulphate to sulphide, because sulphide minerals are not abundant in the iron formations of the Hamersley Basin³. Data²⁶ on the Dales Gorge Member of the Brockman iron formation reveal maximum concentrations of ~ 0.3 wt% and average concentrations much lower. Iron sulphides are relatively insoluble, and any sulphide produced in the sediments by diagenesis would have been precipitated by reaction with the abundant dissolved iron^{27,28}. The apparently minor role of sulphate reduction may have been a consequence of low sulphate concentrations in the

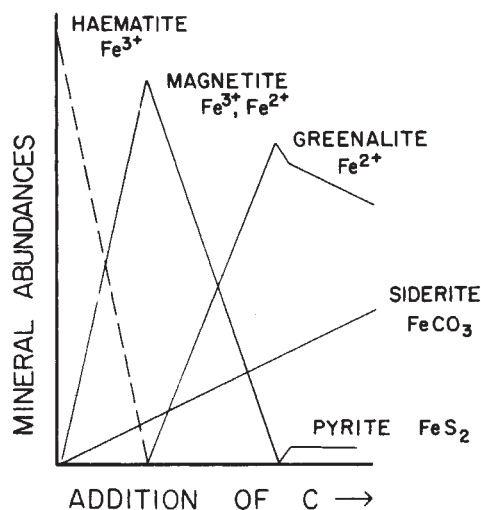


Fig. 1 Suboxic diagenesis of increasing amounts of reduced organic carbon causes the progressive reduction of iron(III) to iron(II). The result is that haematite is replaced by magnetite which is in turn replaced by greenalite and related silicate minerals that contain little or no iron(III). Sulphate reduction and the precipitation of pyrite do not commence until all iron(III) has been reduced. The carbonate produced by diagenetic oxidation of organic carbon precipitates as iron carbonate minerals, siderite and ankerite. The oxide, silicate, and sulphide facies of banded iron formations may reflect increasing amounts of organic carbon in the original sediments.

Archaeon and early Proterozoic ocean²⁹⁻³¹. In recent sediments, when sulphate is exhausted, further diagenetic oxidation of organic matter uses organic matter as the electron acceptor. The metabolic processes are fermentation and methanogenesis. Methanogenesis, however, produces isotopically very light methane and isotopically heavy carbonate²⁴. This process could not, therefore, be the source of the isotopically-light carbonates in the banded iron formations. (For a contrary opinion see ref. 10.)

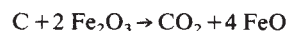
It has recently become clear, however, that iron(III) can serve as electron acceptor in the early diagenetic, bacterial oxidation of organic carbon (see refs 28, 32-34). There is good evidence that particulate iron(III) is reduced to dissolved iron(II) during the anaerobic oxidation by bacteria of reduced organic carbon. The reduction of iron occurs after dissolved oxygen and nitrate have been exhausted and after the reduction of oxidized manganese, but before the reduction of sulphate³². Banded iron formations are, of course, rich in iron. Iron(III) could well have been the electron acceptor that permitted diagenetic oxidation of isotopically-light organic carbon by bacteria to produce the isotopically-light carbonate minerals in iron formations^{10,15}. A stoichiometrically equivalent reaction was proposed by Perry *et al.* as an abiological, metamorphic process.

The initial oxidation of dissolved iron(II) to particulate iron(III) would have occurred in the water column, possibly near the surface. There are several oxidation processes that would not have required significant concentrations of free oxygen. Abiotic photochemical oxidation has been suggested and investigated experimentally^{35,36}. Iron(II) may have served as electron donor in a metabolic process of bacterial photosynthesis³⁷. Alternatively, the electron donor for biological photosynthesis may have been water, with the oxygen produced in the process consumed immediately by reaction with excess dissolved iron⁴.

Consider an iron formation that contains 5 wt% of CO₂ in the form of carbonate minerals²⁶. Suppose that this carbon has an isotopic composition of $\delta^{13}\text{C} = -10\text{‰}$, which represents a mixture of equal parts of oceanic carbonate with $\delta^{13}\text{C} = 0\text{‰}$ and diagenetic carbonate produced by the oxidation of organic matter with $\delta^{13}\text{C} = -20\text{‰}$. The oxidation of 0.7 g of organic carbon per 100 g of rock is required. This would not represent an

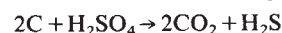
unreasonably large concentration of organic carbon in the initial sediment.

Oxidation of this carbon according to the stoichiometric relationship



would produce 16 g of FeO per 100 g of rock. Iron formations, including those of the Hamersley Basin, typically contain from 20 to 25 wt% of FeO by weight³. The proposed role for early diagenetic oxidation of reduced organic carbon by iron(III) is therefore not contradicted by data on the iron(II) content of banded iron formations.

On the other hand, if 0.7 g of organic carbon were oxidized according to the stoichiometric relationship



the yield would be 0.9 g S per 100 g of rock. The average sulphide concentration of banded iron formations, including those of the Hamersley Basin, is substantially less than this^{3,26}.

What are the implications of an early diagenetic source for much of the carbonate in banded iron formations, a source involving the oxidation of reduced organic carbon and the reduction of oxidized iron(III)? First, because this early diagenetic process is a consequence of the metabolic activities of bacteria, the isotopically light carbonate in banded iron formations may be evidence for the presence of bacteria in the sediments that became banded iron formations³⁸. Microfossils have not yet been discovered in the banded iron formations of the Hamersley Basin³⁸. The calculations presented above suggest that a large fraction of the total iron(II) in these formations may have been reduced by bacteria during early diagenesis. The initial deposits, in this view, contained iron largely in the iron(III) form together with reduced organic carbon in an amount that was stoichiometrically comparable. The preserved ratio of iron(III) to total iron in these sediments is therefore not primary, but is a product of early diagenesis. Also, the generally low concentration of organic carbon in banded iron formations³ is explained as a consequence of the early diagenetic oxidation of an initially much larger amount of carbon by the oxidized iron of the formations themselves. Finally, the oxide, silicate, and sulphide facies of iron formations^{2,39} may reflect a response of the sedimentary system to the early diagenetic alteration of progressively larger amounts of reduced organic carbon (Fig. 1). The oxide facies contains both iron(II) and (III), the silicate facies contains principally the iron(II) form, and the sulphide facies contains iron(II) and sulphide that is presumably the product of bacterial sulphate reduction.

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33,000-yr old chert mining site and related *Homo* in the Egyptian Nile Valley

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The Nazlet Khater 4 site (Nile Valley, Upper Egypt) is located on one of the small wadi-interfluvies in the lower desert near the steep cliffs bordering the western Nile Valley edge (Fig. 1). We have previously reported¹ the excavation of an early Upper Palaeolithic blade industry at this site, although blade industries in the Nile Valley had only been known² to occur since 18,000 yr ago. The 1982 excavation reported here confirms that Nazlet Khater 4 is a chert mining site with a complex extraction strategy, going back 33,000 yr. A nearby grave contained a skeleton of a man in the extended position. We show that the cranial morphology is anatomically modern with archaic characteristics such as a very robust mandible. There is evidence that the skeleton has a similar age to that of the mining site.

The 1982 excavation enabled us to distinguish three sedimentary units: (1) the substratum of the site or the pre-Upper Palaeolithic natural deposits, (2) Upper Palaeolithic diggings in the substratum and their related infills, and (3) later deposits on the site.

The basement of the site consists of greenish silts and fine sands covered by a 1.5-m thick Nile channel deposit, composed of channel lag deposits of well-rounded Nile gravels and chert cobbles and of brown granular silts, covered by a wadi deposit of local limestone gravels (Fig. 2).

Three Upper Palaeolithic digging activities can be distinguished (Figs 1, 2). (1) A trench, 9 × 2 m, with a depth of 1.5 m had been opened at the northern edge of the Nile deposit, just beyond the covering wadi deposit. All the Nile deposits had been removed from this trench which represents ~15 m³ of ground. (2) Vertical shafts, dug down to the channel lag gravel, had sometimes been enlarged at their base to form bell pits. (3) From the walls of the trench or from the bell pits, the channel lag gravels had been extracted from the substratum for several metres, creating short galleries.

The deposits in the trench and in the bell pits consist of two lithological units: gravels at the base, covered by yellow fine aeolian sand. The gravel unit is a purely gravitational deposit, composed mainly of Nile pebbles, mixed with coarse sands, silts, local limestones, Upper Palaeolithic artefacts, and a fragment of gazelle horn core. Several hearths *in situ* with an abundance of charcoal were observed. This gravel unit is heavily encrusted by a 10-cm thick calcrete on its surface. The top of this unit forms a depression and represents an occupation floor with a large hearth.

The overlying infilling of loose aeolian sands completely hides all Upper Palaeolithic diggings. In the trench, three very slightly encrusted layers with gravels and derived artefacts are intercalated¹. During the formation of this unit, important deflation and sand accumulation, not found in the gravel layers, took place. The presence of an *in situ* hearth in this sand unit indicates that the site was occupied during this stage. The post-occupation evolution of the site consists of the formation of a salt crust with desiccation wedges and in the flattening out and re-installation of the overall perfect gently eastward dipping slope.

We have currently nine ¹⁴C dates at our disposal (Fig. 2). Samples GrN-11297, GrN-11300, Lv-1139D, GrN-11301 and GrN-11299 are from different hearths; samples Lv-1142D, Lv-1141D, Lv-1140 and GrN-11296 are from dispersed charcoal. All dates, ranging from 35,100 to 30,360 yr BP, confirm the great age of the mining site. Statistical tests on different combinations of these samples indicate that they do not belong to the same population (significance level better than 99%), so that it is inappropriate to calculate a mean age for the site. It seems justifiable, however, to classify the ¹⁴C dates from the trench according to their lithological positions. The weighted average for the three samples in the gravel unit is 33,470 ± 360 yr BP, and for the three samples in the sand unit, 31,980 ± 650 yr BP. As their significance level is 5%, we suggest that the mining activities were spread over a period of time which can be determined by ¹⁴C dating.

Large quantities of lithic artefacts were recovered. The raw materials are the chert cobbles from the Nile channel lag deposits. Flat elongated chert cobbles were chosen. Flaking of short but regular blades was performed by direct blows with a soft hammer. The butts are flat or punctiform, the bulbs are thin; the Levallois technique was not used.

Tools are rare and are made from flakes or blades by flat retouching, sometimes bifacial, or by oblique retouching. Denticulates are the best represented tools although some burins and end-scrapers are present. Some bifacial axes have been collected; these are flat, made from flakes or blocks, with concave sides and a convex cutting edge (Fig. 3a, b).

The Upper Palaeolithic trenches in the substratum were clearly intended for the extraction of chert cobbles which were subsequently used for flaking. The method of exploitation consisted of undercutting the brown granular silts so that the chert cobbles could be extracted, leaving galleries. This technique mainly took place from the trench. The trench was probably the result of an earlier 'trenching exploitation'³ of the cobble-bearing layer. On the other hand, the trench may have been the result of cleaning after merging different bell pits. It was during the search for the chert cobble-bearing layer, that the bell pits were dug.

The rubble from the trench was dumped on the northeastern edge of the site probably where the chert cobble layer was naturally exposed. We believe that this natural exposure was the reason why the exploitation started.

Such extractions of mineral substances can be considered as real mining³. That this was not continuous mining can be deduced from the fact that prehistoric man was not always sure where to start his digging activities: a shaft was lowered into the rubble from earlier extractions and a search for exploitable chert was carried out in the neighbourhood of the site, such as the site of Nazlet Khater 1, where we excavated a similar bell pit filled with aeolian sand and containing charcoal dated at 31,600^{+3,600}_{-2,500} yr BP (GrN-11298). Mining probably only took place when Upper Palaeolithic man came near Nazlet Khater, where he knew fresh chert to be available.

During the 1980 season a narrow (0.2–0.4 m) grave (1.6 m length and 0.6 m deep) was found on the summit of the boulder hill (Fig. 1). Within the grave was a corpse, lying at full length on its back, with the arms stretched along the body. The covering consisted of several boulders, some more than 0.4 m in diameter. The interstices were filled with loose aeolian sand. A tool was laid on the bottom of the grave, at the right of and next to the

Fig. 1 Site location. 1, Upper Palaeolithic dump area; 2, bell pit; 3, trench; 4, trench wall opening into gallery at the base; 5, excavation limit; 6, chert mining site; 7, enclosed dwelling; 8, grave on the boulder hill.

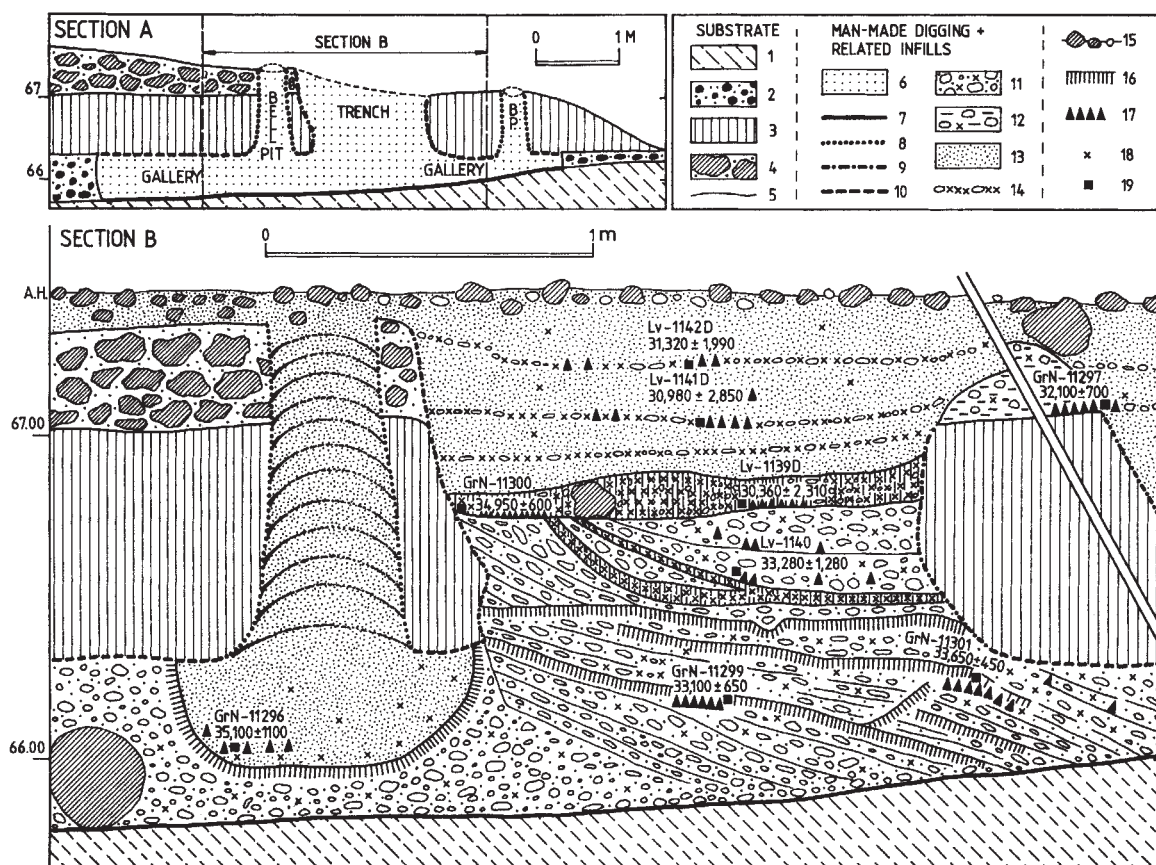
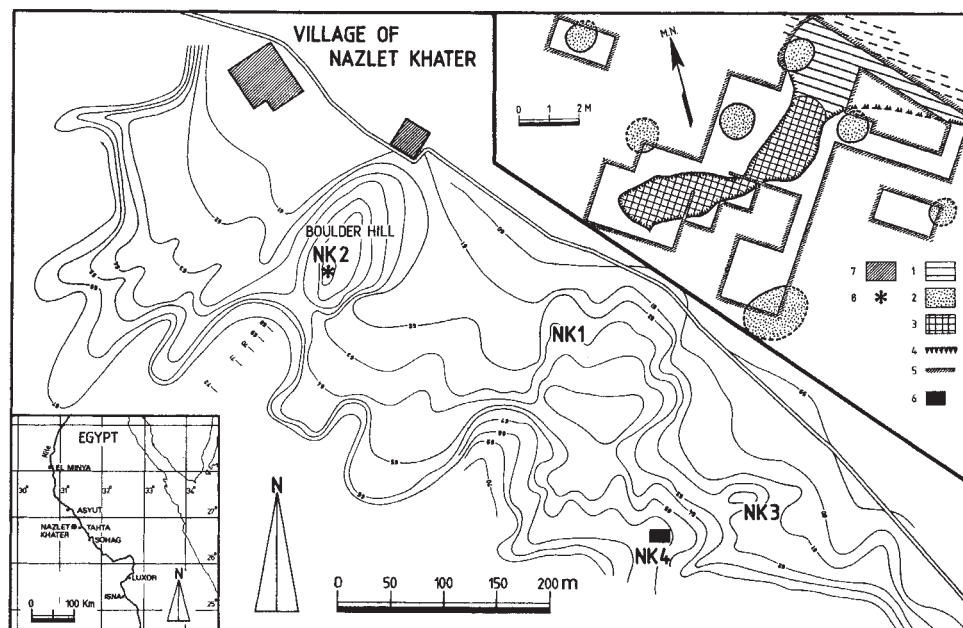


Fig. 2 General (A) and composed (B) section of the site Nazlet Khater. 1, Greenish silts and fine sands; 2, Nile gravels; 3, brown silts; 4, wadi deposits; 5, lithological contacts; 6, extent of the exploitation; 7, base of the mining activities; 8, walls of the bell pit; 9, walls of the trench; 10, gallery roof; 11, man-made gravel infills; 12, surface dumps; 13, aeolian sands; 14, derived gravels and artefacts; 15, desert pavement; 16, calcrete; 17, charcoal; 18, Upper Palaeolithic artefacts; 19, ^{14}C -dates in yr BP.

cranium. It is a bifacially shaped axe with concave sides and a convex cutting edge (Fig. 3c).

From the state of synostosis of most epiphyses and other age indicators, the nearly complete skeletons seems to belong to a sub-adult individual, probably a male, with an estimated height of 1.65 m according to the formulae of Olivier *et al.*⁴. Cranial morphology is certainly that of anatomically modern man. We estimate the cranial capacity to be at least 1,400 cm³. The facial skeleton displays two 'African' characters: fossa praenasalis and

strong alveolar prognatism.

The best-preserved bone is the extremely robust mandible (Fig. 4). Its chin is fully developed. Ramus height (Martin 70)⁵ is 72 mm and ramus breadth (Martin 71) is 51 mm. This breadth is very large and is matched only by that of the Mauer mandible. The rameal index is 70.83. According to its published photograph, the probably Aterian mandible of Dar-es-Soltan-2⁶ (Morocco) must have a rameal index similar to that of the Nazlet Khater man.

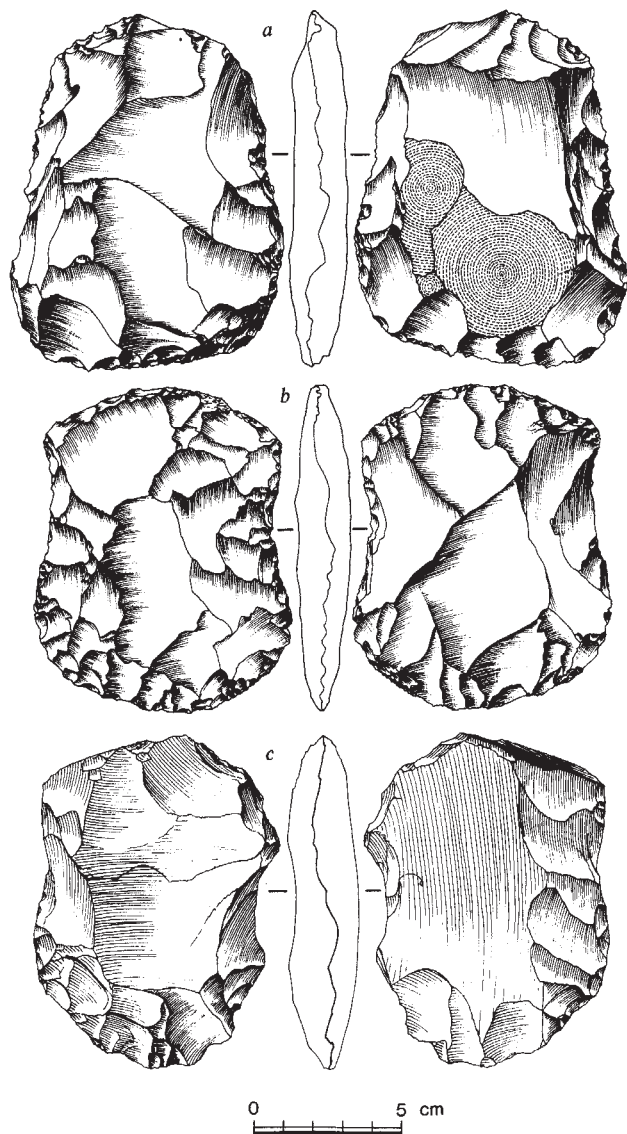


Fig. 3 Upper Palaeolithic axes. a, b, Axes of NK 4; c, burial gift from the grave on Boulder Hill.

The 1982 research shows that the early Upper Palaeolithic blade production on the site of Nazlet Khater 4 is related to chert mining. By about 33,000 yr ago, early Upper Palaeolithic man had acquired typical mining techniques. Indeed, even if the bell pits are only 1.5 m deep, this mining system is comparable with that in general use during the Neolithic⁷. These techniques were so complex that one has to accept that Upper Palaeolithic man chose the bell pit location on the basis of some geological insight.

Extraction pits of raw material to a depth of 0.35 m have been reported from the Nubian Middle Palaeolithic site of Arkin 5⁸. In other parts of the world chert mining was mainly restricted to the Holocene less than 10,000 yr ago. Only ochre extraction⁹ took place before that.

Direct dating of the Nazlet Khater man is not possible as no collagen has been preserved in the skeleton bones. However, several arguments indicate that mining site and grave belong to the same prehistoric technocomplex, which is unique for the Nile Valley, and which was dated at 33,000 yr ago.

Indeed, the bifacial axe found near the cranium of the skeleton in the grave is of the same type as those of the mining site. Such axes are not known to occur in the later Palaeolithic of Egypt and predynastic axes are of a different type. Moreover, we have found no traces of predynastic occupation during our intensive regional survey.



Fig. 4 Mandible (norma lateralis) of the Nazlet Khater man.

The boulder hill grave shows no evidence of the burial practices of the later Palaeolithic and predynastic periods, where the interred body is always in a contracted position.

The only prehistoric trenches filled up with aeolian sand are the grave, the mining site and the dated bell pit on the Nazlet Khater 1 site which suggests that a similar date for the aeolian sand in the grave is likely. A final argument for a very old age is the archaic features of the skeleton itself.

All these arguments strongly suggest that the Nazlet Khater man is around 30,000 yr old. In that case the man of Nazlet Khater is the oldest modern man in Africa north of the Equator.

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The chorus-line hypothesis of manoeuvre coordination in avian flocks

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Thousands of birds flying together at high speeds are able to execute abrupt manoeuvres with such precise coordination that some investigators have postulated that 'thought transference'¹ or electromagnetic communication² must be taking place. Recently, Davis proposed that coordination is achieved by a 'threshold' number of birds executing 'preliminary movements' which signal to the flock that a turn is imminent³. Here I show by analysis of film of dunlin (*Calidris alpina*) flocks that a single bird may initiate a manoeuvre which spreads through the flock in a wave. The propagation of this 'manoeuvre wave' begins relatively slowly but reaches mean speeds three times higher than would be possible if birds were simply reacting to their immediate neighbours. These propagation speeds appear to be achieved in much the same way as they are in a human chorus line: individuals observe the approaching manoeuvre wave and time their own execution to coincide with its arrival.

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The 'chorus-line' hypothesis suggests that manoeuvres are initiated by any bird executing a manoeuvre towards the flock and that subsequent coordination is achieved through visual communication. This leads to the prediction that execution of the manoeuvre by neighbours of the initiator will be delayed by at least their own reaction time but, further away, response times should fall as birds are able to estimate the arrival of the approaching manoeuvre wave. Films taken of human chorus lines indicate that rehearsed manoeuvres, initiated without warning, propagate from person to person approximately twice as fast (107.7 ± 6.8 ms, $n = 3$) as the 194-ms human visual reaction time⁴.

Coordinated manoeuvres (defined as those in which interbird response times were faster than laboratory measured reaction times) were investigated by field observations and analyses of 2,000 ft of slow-motion (50 frames s⁻¹) 16-mm film taken of dunlin flocks at Puget Sound, Washington. Manoeuvres were either natural or were induced artificially by shooting an arrow near an airborne flock. Twenty-two coordinated and four non-coordinated manoeuvres, chosen for clarity of detail, were analysed frame-by-frame. Startle reaction times to a light flash were measured in the laboratory using previously reported methodology⁵.

In films where initiators and all neighbours were discernible, coordinated manoeuvres were initiated by one or a few individuals (one initiator, $n = 9$; two, $n = 3$; three, $n = 2$; >three, $n = 0$). Birds were considered initiators when they began the manoeuvre before the rest of the flock. In all cases coordinated manoeuvres were initiated by birds banking towards the flock ($n = 22$). When flock members turned away from the flock, the flock either did not follow ($n = 16$), or did so at speeds too low to qualify as a coordinated manoeuvre (84.8 ± 15.5 ms, $n = 4$). Manoeuvres always propagated through the flock in a wave radiating from the initiation site. These waves were observed to travel along every major axis (including back to front), indicating that manoeuvres may be initiated from any region of the flock. They had a mean propagation time from neighbour to neighbour of 14.6 ± 6.7 ms ($n = 9$), considerably lower than the laboratory measured startle reaction time of 38.3 ± 3.1 ms ($n = 110$). Therefore, manoeuvres propagate through flocks in waves travelling at speeds nearly three times faster than possible if flock members are following the actions of adjacent neighbours. However, the first neighbours to respond to the initiator required 67 ± 24 ms ($n = 14$). This mean propagation time from initiator to neighbours was significantly slower than both the mean manoeuvre propagation time (14.6 ms, $P < 0.0001$), and the mean species reaction time (38.3 ms, $P < 0.0001$; Wilcoxon rank sum test).

These results show that manoeuvres initially propagate more slowly than the birds' reaction time and then accelerate to high propagation speeds, as predicted by the chorus line hypothesis. Alternative hypotheses make no explicit predictions about the form of propagation, but imply that manoeuvres can occur in unison. No unison manoeuvres were seen. No preliminary movements which might signal that a turn is imminent³ were seen, although such movements should be visible on film if they are to be visible to what is often thousands of tightly packed⁶ flock members.

Flock members always appear to follow the lead of initiators banking towards the flock. This 'dictatorial' rule by initiators presumably prevents indecision and allows flocks to respond rapidly during attacks by birds of prey, which are a major source of mortality for flocking shorebirds⁷. Birds of prey usually direct their attack toward individuals isolated from the main flock^{7,8}, and this may well have exerted selection pressure for the evolution of coordinated manoeuvres.

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Auditory receptive fields in primate superior colliculus shift with changes in eye position

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The process by which sensory signals are transformed into commands for the control of movement is poorly understood. A potential site for such a transformation is the superior colliculus (SC), which receives auditory, visual and somatosensory inputs^{1–3} and contains neurones that discharge before saccadic eye movements^{4–6}. Along the primary sensory pathways, signals coding the spatial location of auditory, visual and somatosensory targets are based on distinctly different coordinate systems, and it is not known whether each type of sensory input uses a separate motor pathway or if they are converted into a common coordinate system in order to share a single pre-motor circuit. Sensory neurones in the SC have spatially restricted receptive fields (RFs) and are organized into maps across the collicular surface^{7–9}. Acute experiments have shown a rough correspondence between the spatial positions of RFs of neurones encountered along a single dorsal–ventral penetration of the colliculus, regardless of the modality of the effective stimulus^{10–14}, suggesting that auditory, visual and somatosensory maps might be in register. However, in these conditions the head-centred auditory system and the retinotopic visual system are aligned because the eyes are in the primary orbital position¹⁵. Moreover, other data have suggested^{16–18} that the primate SC is organized in motor, not sensory, coordinates, although in the cat, eye position was found to have no effect on auditory receptive fields¹⁹. We therefore sought here to determine what happens to the registration of the auditory and visual maps in the alert, behaving animal. Monkeys, with heads fixed, were trained to make delayed saccadic eye movements to auditory or visual targets from one of three initial fixation points while the activity of single neurones was recorded extracellularly. We found that the auditory receptive fields shifted with changes in eye position, allowing the auditory and visual maps to remain in register.

We trained two monkeys (*Macaca mulatta*) to look to either visual or auditory targets in a completely darkened room. The major objective of the experiment was to plot the RFs of sound-sensitive cells in the SC of alert monkeys while varying the direction of visual fixation. We considered that if the RFs of these auditory neurones were organized in head-centred coordinates, this manipulation would have no effect, whereas if the auditory neurones use oculomotor coordinates, the direction and distance of the sound source from the eyes, not the absolute position of the target in space, would be the crucial factor. Accordingly, the magnitude of the neural responses would depend on both the position of the speaker relative to the head and the position of the eyes in the orbit.

Another objective was to determine whether auditory and visual pathways share a common motor circuit. The activity of some collicular neurones is tightly coupled to saccade onset and is thought to participate in the initiation of spontaneous and

visually-triggered saccades⁴⁻⁶. If the neurones that burst before visually-guided saccades also burst before saccades to auditory targets, this would suggest that auditory and visual signals have been converted into the same coordinates by the time they reach the level of the SC.

The monkeys were placed, with their heads fixed, in the centre of a semicircular track²⁰. Under computer control, a speaker moved along the track and the hoop rotated so that the target could be placed anywhere on an imaginary sphere surrounding the animal. A light-emitting diode in the centre of the speaker was used to compare auditory and visual responses. Three fixation lights were placed 24° apart along the horizontal meridian.

The auditory stimulus was a broad-band noise burst (20–20 kHz; 90 dB sound pressure level; re: 0.0002 dyn cm⁻²). The visual stimulus, a green light-emitting diode, subtended a visual angle of 12 arc min. Eye position was monitored using the scleral search coil technique²¹; tungsten microelectrodes were used for extracellular unit recordings.

A delayed saccade task was used to separate temporally sensory and motor activity. At the beginning of each trial, one of the three fixation lights was randomly activated. After a variable time, an auditory or visual target was presented. To receive a liquid reward, the monkey had to maintain fixation of the initial light until it was extinguished and then look to the peripheral target.

For every sound-sensitive cell encountered, the position of the eyes in the orbit had a distinct effect on the neural response to an auditory stimulus. Figure 1*a* illustrates data recorded from a single cell. For these trials, the speaker was located 20° to the right and 6° above the primary eye position. When the gaze was directed towards the left fixation target, the auditory stimulus evoked a vigorous neural discharge with latencies ranging over 40–60 ms. When the monkey was viewing the centre or right fixation lights, identical noise bursts presented in the same spatial location resulted in neural responses that were either markedly attenuated or completely absent.

Figure 1*b* summarizes the data obtained from this cell by plotting the average number of spikes in response to the noise burst against the horizontal position of the speaker (top). The

RF of the neurones shifted with the position of the eyes in the orbit. The peripheral boundary of these RFs could not be determined because of the experimental constraint of keeping the targets within the oculomotor range. These data are replotted using the motor error reference system below. The abscissa represents horizontal motor error, or the horizontal component of the eye movements required to look to the auditory targets from the three fixation points. In this case, the data obtained while the monkey viewed the three fixation lights are closely aligned. Thus, the magnitude of the discharge of this neurone was not determined solely by the position of the auditory stimulus in space, but depended jointly on the position of the eyes in the orbit and the target position. Individual cells in the sample varied with respect to the relative strength of the head-centred and motor error signals they carried, but all auditory units analysed ($n = 116$) displayed a shift in RF location with changes in eye position.

Like many visually responsive cells in the intermediate layers of the SC¹⁶, these auditory neurones could not be classified as strictly sensory or motor cells but seemed to exhibit qualities of both systems. Their responses appeared to be sensory in that the discharge was time-locked to the onset of the stimulus. The average latency was ~50 ms for auditory targets and 70 ms for visual ones. Many of the auditory cells could be activated by either auditory or visual stimuli. For these bimodal cells, the vigour of the neural response depended on target modality, even though identical motor responses might occur. Some cells discharged more vigorously to auditory stimuli, others to a visual target. In an attempt to dissociate the sensory and motor components of the response, we plotted the auditory fields while the monkey performed a probe task—one that required continuous fixation of the initial light without making a saccade towards the auditory or visual probe stimulus. Most cells rapidly habituated when a motor response was not required. For the remaining cells, the RFs obtained using probe trials were very similar to those generated with the delayed saccade paradigm even though no eye movement occurred. Yet this sound-induced activity cannot be termed 'sensory' because the response depended on the trajectory of the eye movement required to look to the auditory target.

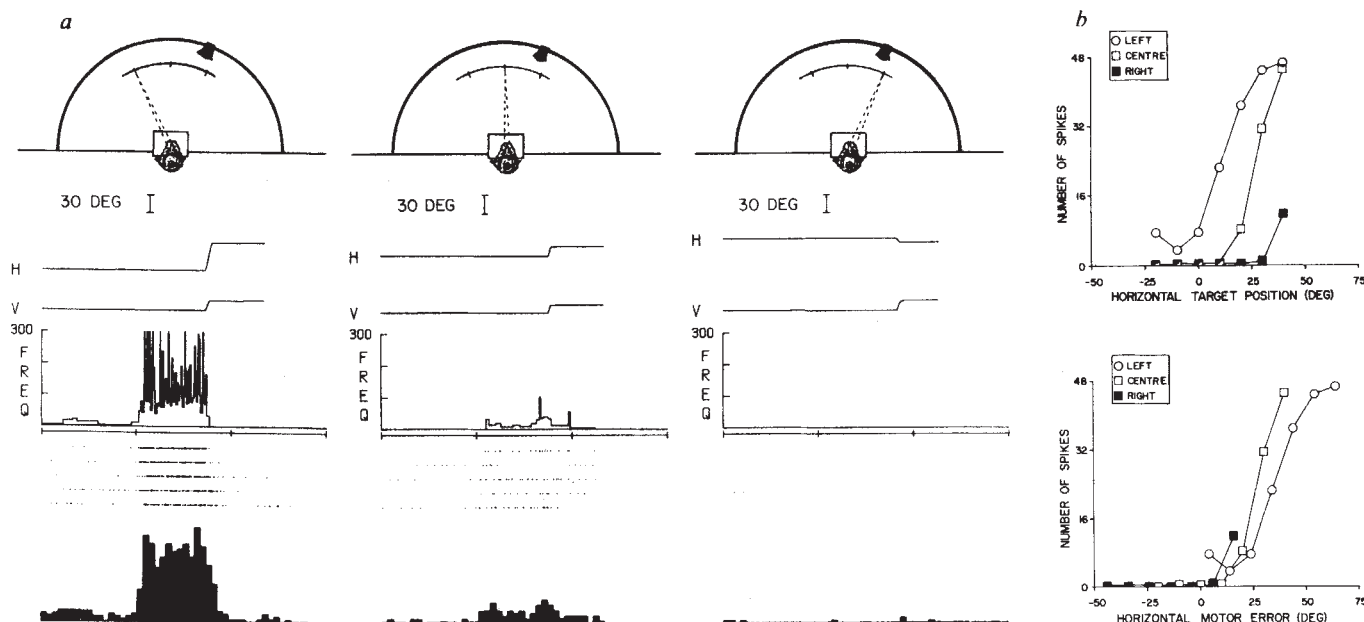
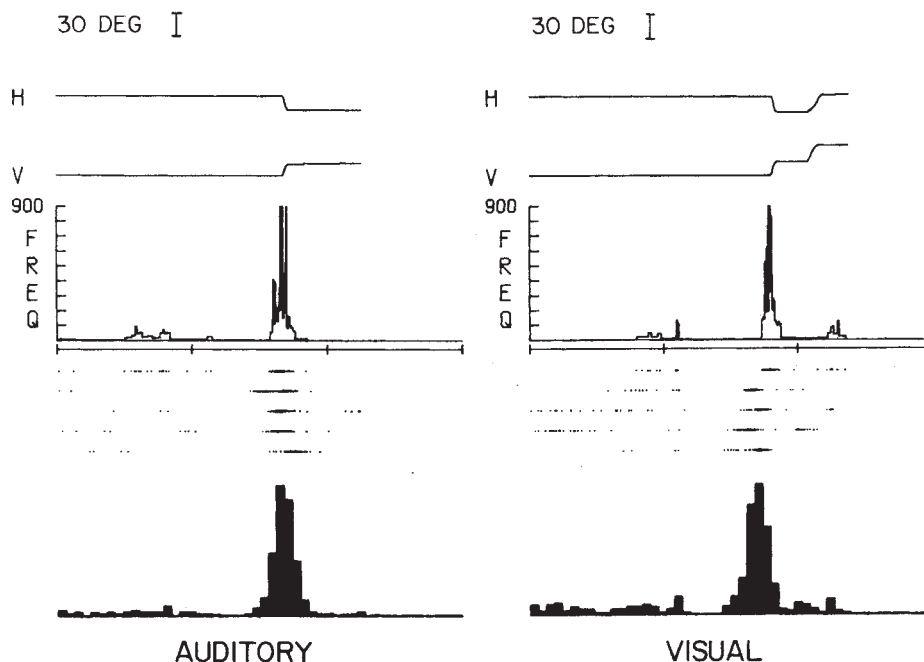


Fig. 1 The effects of eye position on the response of a single SC cell to an auditory stimulus. *a*, The speaker was placed 20° right, 6° up from centre while the fixation position was varied between 24° left (left), centre (centre) and 24° right (right). Time base was 3 s and the target was presented at 1 s. Horizontal (up-right) and vertical eye position traces are shown in the top row. Representations of instantaneous firing rate for single trials are in the second row, followed by rasters showing the unit activity for five trials. The bottom row is a cumulative histogram for these trials. *b*, A summary plot of the auditory RFs for this one cell as a function of horizontal target position (top) showing a shift in the position of the RFs as eye position was varied. The data are replotted below in motor error coordinates—the difference between current eye position and the saccade target. The fields are better aligned in the motor error reference system than in the one based on the position of the target relative to the head.

Fig. 2 The convergence of auditory and visual pathways onto a common motor circuit. A single SR burst cell activated before both auditory (left) and visual (right) saccades to a target located 16° left, 10° up. No burst occurred for either modality when a saccade to this same target was initiated from the left fixation point.



As recent work in the cat SC has shown that the position of the pinnae has a significant effect on the spatial properties of auditory cells²²⁻²⁴, we sought to evaluate the possible contribution of pinna movements in the primate. The RFs were compared in the same cell when the animal was free to move its ears and when the pinnae were mechanically restrained. The field plots were similar in both conditions, indicating that the small pinna movements made by primates are not responsible for the eye position effect observed in this study.

SC neurones that burst before visually initiated saccades were also found to burst before saccades to auditory targets. The activity of a typical saccade-related (SR) burst neurone is illustrated in Fig. 2. With gaze directed at the centre fixation SR burst cells tested in this manner. Thus, at the level of the SC, auditory and visual signals share a common motor circuit.

Our findings suggest that an internal representation of eye position, either efference copy or proprioception, has been subtracted from the head-centred spatial code of auditory targets found elsewhere in the auditory system. As a result, some auditory cells code for the vector of a potential eye movement rather than just the position of a sound source relative to the head. The fact that the auditory cell population we observed displayed a range in the relative potency of motor error and head-centred signals suggests that this transformation may occur in the primate SC. This translation allows the auditory and visual maps in the intermediate and deep layers of the primate SC to share a reference system (motor error) and to converge onto a common motor pathway for the generation of saccadic eye movements.

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Association with persistent neuroleptic-induced dyskinesia of regional changes in brain GABA synthesis

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The movement disorder tardive dyskinesia is a serious side effect of the long-term treatment of schizophrenia with neuroleptic drugs. Similar symptoms to those of tardive dyskinesia have been observed in *Cebus apella* monkeys following long-term treatment with neuroleptic drugs^{1,2}, and these monkeys may therefore be a useful animal model of tardive dyskinesia. Motor defects have persisted in these dyskinetic monkeys for periods of 1-6 yr after the cessation of neuroleptic treatment. We report here that in three regions of the brains of dyskinetic monkeys (substantia nigra, medial globus pallidus and subthalamic nucleus) glutamate decarboxylase activities and γ -aminobutyric acid (GABA) levels are reduced relative to control monkeys that had been treated with neuroleptics but which showed none of the symptoms of tardive dyskinesia. These results suggest that alterations in the GABA neurone system are involved in neuroleptic drug-induced tardive dyskinesia.

Nineteen *C. apella* monkeys (10 male, 9 female) weighing 1.6–4.2 kg (mean 2.9 kg) were used in the present experiments. Twelve monkeys received chronic neuroleptic treatment: haloperidol (nine animals) or fluphenazine (three) for 3–6 yr, while seven were untreated controls. Intramuscular (i.m.) injections of haloperidol decanoate (1–10 mg per kg) or fluphenazine decanoate (4–10 mg per kg) were given at 3-week intervals. After varying treatment periods, six of these monkeys (three injected with haloperidol, three with fluphenazine) developed persistent choreic dyskinetic movements in the trunk, neck and extremities (one monkey also had oral dyskinesia). The frequency of dyskinetic movements and symptom intensity were rated during 2-min sessions³. When these measurements were made at 1 and 60 days after the final neuroleptic injection, only slight changes in intensity of dyskinetic symptoms were evident.

Throughout the present study three groups of animals were compared: untreated controls ($n = 7$), neuroleptic-treated controls ($n = 6$) and dyskinetic animals ($n = 6$). Before the neuroleptic-treated animals were killed the regular depot drug was substituted by a single i.m. injection of non-depot haloperidol or fluphenazine (0.05 mg per kg). Two months after the final injection, the animals were killed under brief methohexital anaesthesia. Within 10–12 min of killing the brains were removed and stored at -80°C . Microdisks (1.075 mm^3) were later punched from successive coronal sections prepared from the frozen brains. Glutamic acid decarboxylase (GAD) activity was measured as $^{14}\text{CO}_2$ formation from $[1-^{14}\text{C}]$ glutamic acid during 30 min in the presence of pyridoxal-5-phosphate⁴. GABA, homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were assayed by gas chromatography–mass spectrometry in the selected ion-monitoring mode using deuterium-labelled internal standards^{5,6}. In most animals only the left half of the brain was analysed, but in six (two untreated controls, one neuroleptic-treated control and three dyskinetic monkeys) both sides were compared (one of the dyskinetic monkeys had one-sided symptoms).

Mean levels of transmitter markers at each brain site were compared between the three groups of monkeys using independent Student's t -tests. In addition, all values obtained for a certain brain nucleus were combined and means for each nucleus were compared using t -tests between the three groups. In one case (see Fig. 1) sign-tests and χ^2 -tests were used.

The neuroleptic-treated monkeys which did not develop dyskinesia (neuroleptic-treated controls) did not differ significantly

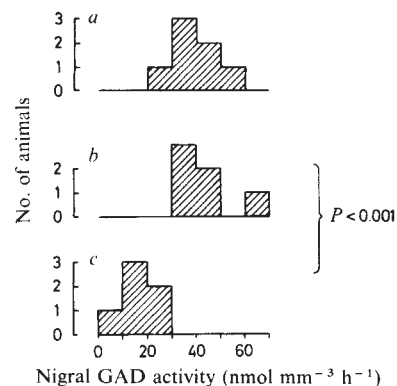


Fig. 1 Distribution of individual (mean) GAD activity levels in substantia nigra in three groups of monkeys: *a*, untreated controls; *b*, neuroleptic-treated controls; and *c*, dyskinetic animals.

from untreated controls in any brain region with regard to GAD activity and levels of GABA, HVA or DOPAC, whereas the dyskinetic animals showed a characteristic pattern of regional changes. Table 1 shows regional GAD activity and GABA levels for untreated controls and per cent changes within both neuroleptic-treated groups. The dyskinetic monkeys showed a reduced GAD activity in the substantia nigra, nucleus subthalamicus and medial globus pallidus and GABA was low in the substantia nigra and n. subthalamicus. In all other brain areas group differences for GAD and GABA were insignificant. In Fig. 1 the mean nigral GAD activity for each individual monkey has been plotted against the number of animals. The dyskinetic group differed from the controls (χ^2 tests, $P < 0.001$), verifying that the number of animals was sufficiently large to allow generalized conclusions regarding relations between state (dyskinetic versus non-dyskinetic) and biochemical changes.

GAD activity in substantia nigra was generally equally high on both sides; a conspicuous exception was found in one animal with unilateral dyskinesia in the left hand and arm. In this animal there was a depression of GAD activity only in the opposite (right) nigral area ($15.3\text{ nmol mm}^{-3}\text{ h}^{-1}$), whereas the ipsilateral nigra had a mean value in the normal range ($41.0\text{ nmol mm}^{-3}\text{ h}^{-1}$).

The HVA levels were significantly reduced in the caudate nucleus and substantia nigra of dyskinetic animals (Fig. 2). In

Table 1 Regional brain GAD activity and GABA concentrations ($\pm$ s.d.) in seven untreated controls, and per cent deviations in six neuroleptic-treated controls and six monkeys with neuroleptic-induced persistent dyskinesia

Area	GAD			GABA		
	nmol $\text{mm}^{-3}\text{ h}^{-1}$ UC	% Δ NC	% Δ NPD	ng mm^{-3} UC	% Δ NC	% Δ NPD
Cortex	9.9 ± 1.6	-7	-5	255 ± 35	-10	-11
Caudate	11.7 ± 2.1	+4	0	328 ± 93	-3	-6
Putamen	12.2 ± 1.5	-1	0	393 ± 56	-6	-6
Accumbens	19.2 ± 2.6	+9	+5	791 ± 25	0	-6
Globus pallidus lateralis	36.5 ± 7.0	+2	-16	$1,210 \pm 42$	-5	-9
Globus pallidus medialis	36.9 ± 2.4	+4*	-26‡	$1,420 \pm 26$	-5	-5
Amygdala	8.6 ± 0.9	-13	-12	287 ± 53	-10	-8
Hypothalamus	21.5 ± 4.4	-3	-8	735 ± 97	-9	+2
Thalamus	9.8 ± 1.7	-4	-4	322 ± 59	+2	+3
Nucleus subthalamicus	9.7 ± 2.3	+5*	-32§	504 ± 79	-20	-47‡
Substantia nigra	45.8 ± 6.6	+18†	-57	$1,000 \pm 133$	-9	-20‡
Nucleus ruber	8.9 ± 1.9	+17	+19	374 ± 37	-3	+2
Geniculate lateralis	8.4 ± 1.0	+4	-11	196 ± 21	+10	+4
Superior colliculus	13.4 ± 2.9	-18	+10	545 ± 39	-3	-4
Formatio reticularis	18.0 ± 3.9	-7	-5	612 ± 29	-10	-9
Pons	10.5 ± 5.1	-10	-17	379 ± 147	0	-8

UC, Untreated controls; NC, neuroleptic-treated controls; NPD, monkeys with neuroleptic-induced persistent dyskinesia. The NC group did not significantly differ from UC at any brain site.

* $P < 0.01$; † $P < 0.001$, NPD compared with NC.

‡ $P < 0.05$; § $P < 0.01$; || $P < 0.001$, NPD compared with UC.

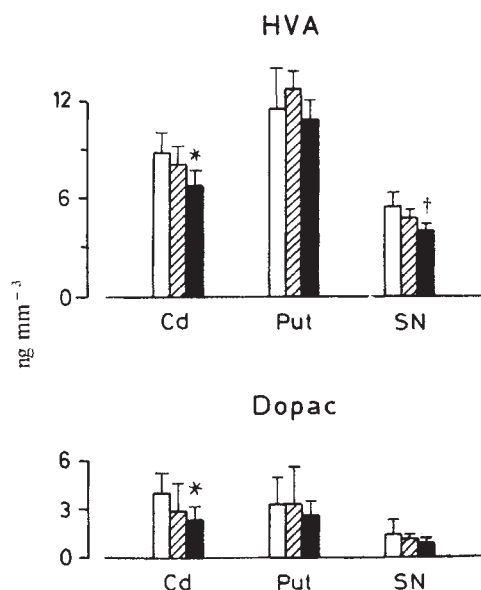


Fig. 2 HVA and DOPAC levels in the caudate nucleus (Cd), putamen (Put) and substantia nigra (SN) of seven untreated controls (open columns), six neuroleptic-treated controls (hatched columns) and six dyskinetic monkeys (solid columns). Values plotted are means $\pm$ s.d. Significance levels: * $P < 0.05$, † $P < 0.01$.

the caudate there was also a reduction of DOPAC. In all striatal areas studied (20 in the caudate, 16 in putamen) the mean levels of transmitters in dyskinetic animals were lower than in neuroleptic-treated controls. The greatest depression was found in corpus nuclei caudati and in some nigral areas (unfortunately the technique did not allow us to differentiate between nigra reticulata and compacta).

When striatal dopamine transmission is blocked by neuroleptic drugs this, by trans-synaptic mechanisms, elicits a series of changes in activity elsewhere in the brain⁷. The turnover of GABA in the substantia nigra is lowered⁸, which in turn has effects on nigral efferents under tonic inhibitory control from the striatonigral GABA neurone system⁹⁻¹². In the present experiments we noted a lowered GAD activity and/or reduced GABA levels in three brain areas: the substantia nigra, globus pallidus medialis and the subthalamic nucleus. These signs of a reduced GABA function in three brain nuclei were evident only in dyskinetic animals and are probably due to longstanding local depression of GABA neurone activity. Thus, even if all monkeys display local GABA depression during neuroleptic drug administration, only the dyskinetic animals fail to recover their GABA function after withdrawal of the drug.

Further evidence of a connection between dysfunction of nigral GABA and movement disorders is provided by the observation that intra-nigral injection of GABA antagonists precipitates oral dyskinesia in rats¹³. In this context it is noteworthy that low nigral and striatal levels of GABA and GAD have been reported in patients dying of Huntington's chorea, another chronic dyskinetic condition^{14,15}. Low nigral GAD activities have also been reported in subjects suffering from Parkinson's disease—again this is secondary to the impaired striatal dopamine transmission, since L-dopa seemed to retain nigral GAD activity at normal levels¹⁶. Analogously, intra-striatal injection of dopamine has been reported to increase glucose utilization in those areas (substantia nigra, globus pallidus medialis and subthalamic nucleus) where we noticed a reduction of GAD activity in our dyskinetic monkeys¹⁷. A

reduction of nigral GAD activity has also been found in rats made dyskinetic by chronic haloperidol administration⁴. GAD is known to be preferentially located in GABA neurone terminals¹⁸. Whether the low GAD activity in the three brain areas reported here is due to a reduced tissue density of GABA terminals (that is, caused by neuronal cell degeneration) or a reversible functional depression, must await histological evidence.

The reduced nigral and striatal dopamine metabolism in dyskinetic monkeys would seem incompatible with a dysfunctioning striatonigral GABA system if this is regarded as a link in a negative feedback control of nigrostriatal dopamine nerves. If this feedback loop hypothesis¹⁹⁻²³ were correct, an impairment of its GABAergic link would be expected to increase rather than reduce the turnover rate of dopamine. However, this hypothesis has been disputed and the possibility of a positive feedback has occasionally been proposed²⁴. The finding that GABA agonists injected into the substantia nigra reticulata induce contraversive turning and an increased turnover of dopamine in the striatum^{12,25-28} supports the notion that dopamine neurones are subjected to positive feedback.

Supersensitivity in striatal dopamine receptors has been suggested as the main cause of tardive dyskinesia^{29,30}. However, when rats are given neuroleptic drugs they develop such supersensitivity to dopamine agonists even after a single injection³¹. Further, this phenomenon is not irreversible and thus poorly if at all related to dyskinetic movements continuing for months after neuroleptic drug withdrawal. The present paper instead seems to link persistent dyskinesia with a weakness in the GABAergic neurone system induced by trans-synaptic mechanisms in neurones remote from the striatal dopamine receptor.

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Chronic neuroleptic treatment enhances neurotensin receptor binding in human and rat substantia nigra

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Antischizophrenic neuroleptic drugs interact with brain dopamine systems in producing both therapeutic and unwanted side effects¹. Short-term administration of neuroleptics may produce a parkinsonian, hypokinetic syndrome mimicking symptoms of dopamine depletion, and presumably caused by dopamine receptor blockade². Paradoxically, some 20% of patients treated chronically with these agents develop tardive dyskinesia. The adventitious movements typical of this condition mimic symptoms of dopamine excess despite continuing receptor blockade³⁻⁵. Dopamine receptor supersensitivity does develop in such conditions, but its extent does not correlate well with the presence or absence of tardive dyskinesia⁶. Exploration of neuroleptic-induced alterations in other dopamine-associated systems may thus provide insight into these processes. Dopamine-containing cells of the substantia nigra, prominently implicated in the motor side effects of neuroleptics, possess dense concentrations of receptors for the putative peptide neurotransmitter, neurotensin⁷⁻⁹. Here we report that these receptors are substantially increased in both rats and humans after chronic treatment with neuroleptic drugs, and discuss possible implications of this finding for our understanding of neuroleptic actions.

Neurotensin is a central nervous system neurotransmitter candidate anatomically disposed to interact with dopaminergic neurones¹⁰⁻¹³. In particular, several lines of evidence indicate that there are dense localizations of neurotensin receptors on neurones in the substantia nigra from rats and human tissue. Autoradiographic studies demonstrate dense concentrations of neurotensin-binding sites in the substantia nigra that are depleted by rat and human lesions destroying dopamine-containing cells^{7-9,14}. The neurotensin receptors appear to be physiologically active. Intracisternal neurotensin administration depletes striatal dopamine levels¹⁵, while microiontophoretic application of the peptide selectively excites nigral compacta neurones¹⁶. Neurotensin potently enhances dopamine release from striatal slices¹⁷. We have examined these nigral neurotensin receptors in rats and humans with and without chronic neuroleptic (haloperidol) treatment.

Sprague-Dawley rats (200 g) were injected subcutaneously (s.c.) daily for 4 weeks with 0.5 mg per kg of either haloperidol or vehicle alone, according to the regimen of Burt *et al.*¹⁸. Frozen midbrain slabs taken from brains of drug-treated patients with schizophrenia and from neurologically normal controls (given by Drs Wallace Tourtellotte and Peter Whitehouse) were maintained at -70 °C until sectioning (see Fig. 3 legend for details). Neurotensin receptor autoradiography was performed as described elsewhere⁷. Briefly, a 60-min incubation with 4 nM ³H-neurotensin (86 Ci mmol⁻¹; NEN) was followed by two 5-min washes, then rapid drying of the tissue. Serial sections incubated in medium containing 5 × 10⁻⁶ M unlabelled neurotensin (Peninsula) and ³H-neurotensin served as 'blank' controls. Autoradiography was performed using the emulsion-coated coverslip technique (Kodak NTB 3). After 6 weeks of exposure, the emulsion was developed and the tissue stained with toluidine blue. For 'saturation' analysis, sections from two haloperidol-treated rats, two control rats, two neuroleptic-treated schizophrenics, and two neurologically normal control patients were incubated in 10⁻⁷ M labelled neurotensin, as

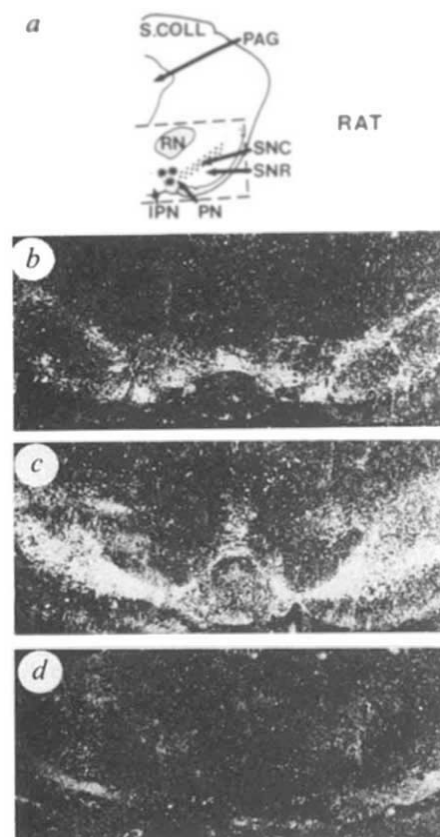


Fig. 1 Neurotensin receptors in rat basal midbrain. *a*, Diagram of the midbrain area shown in *b-d* (the dashed box represents the left half of these photographs). SNR, substantia nigra pars reticulata; SNC, substantia nigra pars compacta; IPN, interpeduncular nucleus; RN, red nucleus; PAG, periaqueductal grey; PN, nucleus paranigralis; S. COLL, superior colliculus. *b*, Darkfield photomicrograph of ³H-neurotensin autoradiogram of the basal midbrain from a control saline-injected rat. *c*, Same as *b* for haloperidol-injected rat. *d*, 'Blank' section adjacent to *a*, incubated with 5 × 10⁻⁶ M unlabelled neurotensin and 4 nM ³H-neurotensin to establish a control. ×32.

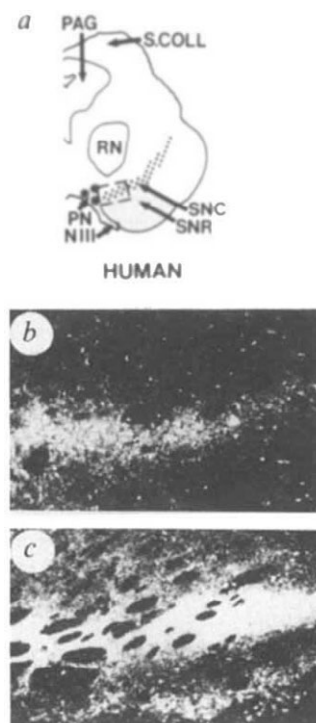


Fig. 2 Neurotensin receptors in human basal midbrain. *a*, Diagram of the midbrain area shown in *b, c*. (Box represents the approximate location of the photographs; it is smaller here than in Fig. 1 as the human nigra is ~5 times larger than the rat nigra.) NIII, oculomotor nerve. Other abbreviations as for Fig. 1. *b*, Darkfield photomicrograph of ³H-neurotensin autoradiogram of the basal midbrain from a neurologically normal human. *c*, Darkfield photomicrograph of ³H-neurotensin autoradiogram of the basal midbrain from a schizophrenic, neuroleptic-treated human. Broadening of the zone of dense neurotensin receptor grains is evident in the human nigra, as in the rat nigra, following neuroleptic treatment. ×36.

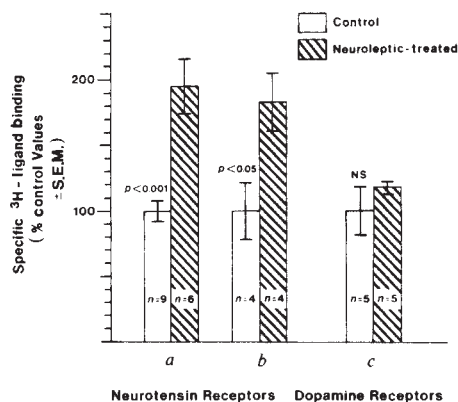


Fig. 3 Histogram of neurotensin (a and b) and dopamine (c) receptor densities in substantia nigra of normal and neuroleptic-treated humans (a) and rats (b and c). a, Specific ^3H -neurotensin receptor binding levels in substantia nigra specimens from neuroleptic-treated schizophrenic patients and neurologically normal humans without known neuroleptic treatment. Mean age of the control patients was 51; that of the schizophrenics 50. Mean postmortem interval for control brains, 22 h; for schizophrenics, 29 h. The age, postmortem interval, disease duration, cause of death and medication regimen of the six drug-treated human schizophrenic patients were as follows: S1: 36 yr, 27 h; >15 yr, ? overdose, fluphenazine/thioridazine/doxepin/molindon. S2: 36 yr, 41 h, ?15 yr, gunshot wound, chlorpromazine/thiothixene/fluphenazine/trifluoperazine. S3: 46 yr, 24 h, 20 yr, myocardial infarction, fluphenazine. S4: 51 yr, 29 h, "years", aspiration, loxapine. S5: 64 yr, 39 h, 12 yr, bowel perforation, chlorpromazine/trifluoperazine. S6: 67 yr, ~12 h, "years" ?overdose, ?haloperidol. The age, postmortem interval and cause of death for nine neurologically normal control patients without histories of neuroleptic use were as follows. N1: 30 yr, 33 h, multiple trauma. N2: 36 yr, 19.5 h, myocardial infarction. N3: 40 yr, 42 h, myocardial infarction. N4: 40 yr, 19 h, sudden death. N5: 42 yr, ~18 h, myocardial infarction. N6: 61 yr, 23 h, myocardial infarction. N7: 65 yr, ~12 h, myocardial infarction. N8: 66 yr, 16 h, gunshot wound. N9: 77 yr, ~12 h, ruptured aorta. b, Specific ^3H -neurotensin receptor binding levels in substantia nigra specimens from rats treated for 1 month with 0.5 mg per kg per day of either haloperidol s.c. or vehicle alone. c, Dopamine receptor binding (ADTN (2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene)-displaceable ^3H -spiroperidol binding) to rat substantia nigra sections adjacent to those assayed for ^3H -neurotensin binding in b. (Data from G.R.U., P. Whitehouse, D. Price and M.J.K., in preparation.)

described above, and exposed to coverslips for 3 weeks. Dopamine receptors were labelled as described elsewhere¹⁹. We counted grains overlying the substantia nigra zona compacta, ~400 μm from its medial border. Three 1,000- μm^2 regions of each substantia nigra were counted manually in each case. The data presented here derive from the substantia nigras of nine normal humans, six drug-treated schizophrenic patients, five saline-injected rats and five haloperidol-injected rats. Values for adjacent 'blank' sections from these same regions were subtracted from the values obtained to yield 'specific' grain counts. Statistical calculations (Student's *t*-test, Pearson correlation coefficient) were performed using a Hewlett-Packard programable calculator.

In the control human brains, and in rat brains, receptor distributions in the basal midbrain were similar to those reported previously⁷⁻⁹. High densities of receptor grains overlying the substantia nigra and ventral tegmental area contrasted with much lower levels over the cerebral peduncles and red nucleus (Figs 1b, 2b). These high grain densities were 6-10 times the levels noted in adjacent 'blank' sections incubated in the presence of unlabelled neurotensin (Fig. 1d). Values obtained for human blank samples were also less than one-sixth of total nigral binding. Densities of receptors in human control cases

did not correlate significantly with either patient age or postmortem interval ($P > 0.1$ in each case).

Binding of labelled neurotensin in the substantia nigra was substantially increased in both rats and humans treated with neuroleptics (Figs 1c, 2b). There was almost a doubling of receptor densities in both species, with only slight overlap between normal and drug-treated values ($P < 0.05$ for rats; $P < 0.01$ for humans) (Fig. 3a, b).

Saturation analysis suggested that this alteration in receptor binding was principally due to alterations in receptor number, as we found the same relationship between treated and untreated individuals. Human schizophrenic tissue showed 1.8 times the binding in normal control tissue, while drug-treated rats revealed 1.9 times, however, control binding. Our ability to detect subtle changes in the affinity constant, K_D , using this paradigm is, however, limited.

Could these results be due to direct neuroleptic influences on peptide binding? In *in vitro* binding assays performed using homogenates or tissue sections, concentrations of haloperidol up to 10^{-5} M were ineffective in displacing or augmenting neurotensin binding, making this an unlikely possibility (G.R.U., unpublished data)¹⁰.

Recently, Mazella *et al.* described²⁰ high- ($K_D = 0.1$ nM) and low- ($K_D \sim 4$ nM) affinity binding sites for ^{125}I -neurotensin; we might expect ~50% occupancy of lower affinity and ~97% occupancy of higher affinity sites in our binding conditions. It is possible that we have labelled both of these sites in our studies²⁰.

These results may have implications for understanding receptor regulation, and might provide insight into neuroleptic drug effects. Chronic administration of neuroleptic, dopamine receptor-blocking drugs has been associated with several brain changes. Nigrostriatal receptors for dopamine, especially the D-2 subtype, and for cholecystokinin peptides are increased by ~20% by neuroleptics^{6,18,21}. We have observed similar quantitative changes in autoradiographic dopamine receptor binding in our rat brains, although these alterations did not reach statistical significance with our small sample (G.R.U., P. Whitehouse and M.J.K., in preparation; Fig. 3c). In the present study, neuroleptic-induced neurotensin receptor changes were of substantially greater magnitude than alterations in the density of other receptors influenced by these drugs. Recent electron microscopic studies revealing increased numbers of terminals on nigral dendrites in neuroleptic-treated animals could provide a possible anatomical correlation with the present results²². Interestingly, the increased population of nerve endings possesses properties characteristic of (though not diagnostic of) peptide-containing terminals²³⁻²⁶. Conceivably, presynaptic as well as postsynaptic peptidergic mechanisms could be involved. Neurotensin levels are elevated in some brain areas in neuroleptic-treated rats and schizophrenics, though nigral peptide levels have not been examined^{27,28}.

These results seem to provide an example of 'autoregulatory' responses extending beyond changes in drug-blocked receptors to include other receptor types on the same cell. The increased density of neurotensin receptors could lead to increased excitability of dopamine cells, and partially supercede the effects of dopamine receptor blockade. Whether such an effect could occur at the level of the individual cell, or whether it requires polysynaptic feedback circuits such as those provided by striatonigral projections awaits further study.

The magnitude of the neuroleptic-induced neurotensin receptor change and its occurrence in both rat and human brains are consistent with a possible role for these peptide receptor alterations in the clinical expression of long-term neuroleptic effects such as tardive dyskinesia. Augmented neurotensin receptor densities on nigrostriatal neurones could possibly lead to increased dopamine release from these neurones, and hence to motor symptoms of dopamine excess. Conceivably, elucidation of neuroleptic-induced alterations in nigral neurotensin receptors could enhance our understanding of the motor side effects of frequently used neuroleptic drugs.

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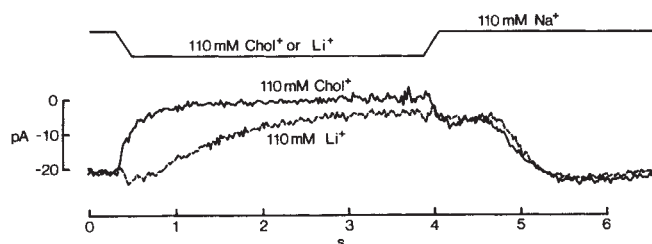


Fig. 1 Effects on the light-sensitive current in toad rod of complete replacement of Na in Ringer's by choline and Li. External Ca was maintained at 1 mM throughout. Recordings were made from the same rod with a current-to-voltage converter connected to the suction pipette. Time courses of solution changes, as indicated by junction currents, are shown above the traces.

Methods: The valve which controlled solution changes was operated pneumatically and with electronic timing so that the solution changes could be reproduced exactly many times. Traces were obtained from total recorded current (light-sensitive current plus junction current), measured in darkness, minus junction current, measured in a repeated run in bright light. Outward light-sensitive current across the outer segment is plotted upwards. In addition to 110 mM Na, choline or Li, the solutions also contained 2.5 mM K, 1.6 mM Mg, 1.0 mM Ca, 117.7 mM Cl, 5 mM tetramethylammonium (TMA)-HEPES, 5 mM dextrose and were buffered to pH 7.65. The choline trace is averaged from two dark minus two light trials; the Li trace is from the difference between single dark and light trials. All recordings were low-pass filtered at 100 Hz, 8-pole, and bright steady light for junction current measurements delivered 80–189 photons (500 nm) $\mu\text{m}^{-2} \text{s}^{-1}$, unpolarized.

study the light-sensitive conductance before it collapsed upon removal of all external Na^{14} .

The solid trace in Fig. 1 shows an experiment in which all Na in the perfusing Ringer's was briefly replaced by choline, a cation with little or no ability to go through the light-sensitive conductance^{13,14,18}. The initial rapid reduction in current upon Na removal indicates removal of most or all of the Na current, while the small initial current jump upon restoring Na probably represents Na current going through a substantially lower light-sensitive conductance caused by the failure of choline to drive the Na–Ca exchanger and maintain open conductance. The delayed secondary increase in current probably reflects the gradual reopening of the conductance due to the reactivation of the Na–Ca exchanger by Na, which leads to lower internal Ca. The dashed trace in Fig. 1 was obtained from the same cell with Li replacing Na. There was no initial drop but rather a slight increase in current upon Li substitution, but thereafter the current did decline to a low level. Upon restoring Na there was no initial jump in current, but the secondary rise was identical to the choline case. These results suggest that Li goes through the light-sensitive conductance as well as or slightly better than Na but that it cannot run the Na–Ca exchanger. In the squid axon Li also cannot substitute for Na in driving the exchanger¹⁹.

Figure 2a shows a similar experiment in which K completely replaced Na. Like the Li case, there was an excess of inward light-sensitive current compared with choline replacement that could only be attributed to K. The small initial decline in current indicates that the conductance is somewhat less permeable to K than to Na, while the secondary decline again suggests a progressive reduction in the conductance. From the initial current reductions in choline and in K the permeability ratio K:Na was estimated to be about 0.6. Besides carrying current, external K also tended to reduce the light-sensitive conductance. Figure 2b shows that when 20 mM or 55 mM Na was replaced, the steady-state current was actually smaller in K than in choline, even though K could carry substantial current. This blocking action of K seems to arise from inhibition of the Na–Ca

Cation selectivity of light-sensitive conductance in retinal rods

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Vertebrate photoreceptors respond to light by a membrane hyperpolarization which is thought to result from the decrease of a Na-selective conductance in the outer segment^{1–6}. One hypothesis is that light increases intracellular free Ca which reversibly blocks the Na conductance^{7,8}; at least part of this Ca is then extruded to the cell exterior by a Na–Ca exchanger at the plasma membrane^{9–14}. We describe experiments here which show that the light-sensitive conductance in rods is also highly permeable to K. While external Na acts to keep the conductance open, external K tends to keep it closed, both actions probably involving the Na–Ca exchanger. The conductance is also permeable to the monovalent cations Li, Rb and Cs and the divalent cations Ca, Sr and Ba. The ability of both Na and K to go through the light-sensitive conductance explains the long-standing puzzle as to why the reversal potential for the light response is at 0 to +10 mV^{15–17}.

Under IR light a piece of retina from a dark-adapted toad (*Bufo marinus*) was finely chopped in Ringer's solution, yielding some isolated rods with intact outer and inner segments. The light-sensitive current from one of these rods was recorded by sucking its inner segment into a close-fitting glass pipette containing Ringer's solution while its outer segment was exposed to perfusing solution^{13,14}. The solution exchange time as judged from junction currents¹⁴ was about 200 ms, rapid enough to

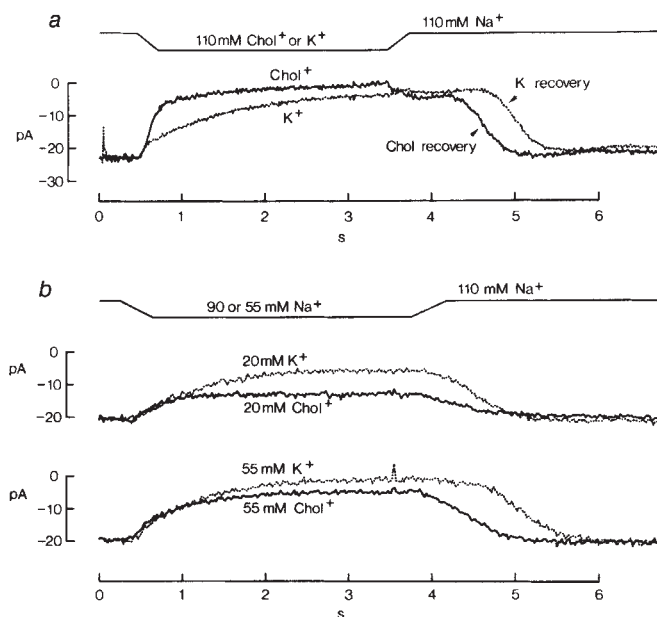


Fig. 2 Effect on rod light-sensitive current of the replacement of Na in Ringer's by K. 1 mM external Ca throughout. *a*, Complete replacement. *b*, Partial replacements. Two separate cells in *a* and *b*. The junction currents are already subtracted in each trace. All other ionic concentrations are the same as in Fig. 1 (including the extra 2.5 mM K). In *a*, the choline record is averaged from the differences between two dark and two light trials (alternated) while the K record is averaged from the differences between four dark and four light trials. In *b*, each of the choline and K records is averaged from the difference between two dark and two light trials. Small spikes in lower records of *b* are from incomplete subtraction of valve movement artefacts. Temperature 24.5 °C in both cases.

exchanger, as suggested by the increased delay in recovery after high K exposure (Fig. 2*b*). Indeed, the recovery after 55 mM Na, 55 mM K was as much delayed as that after 0 Na, 110 mM choline (see Fig. 2*a*), when the Na-dependent Ca efflux is presumably fully inhibited. Part of this K inhibition is probably due to a membrane depolarization effect on the exchanger, resulting from high K leaking into the pipette and affecting the inner segment, but there may also be a direct inhibition by K on the exchanger.

It has previously been shown that low external Ca substantially increases the light-sensitive current in rods^{7,13,14}, apparently by reducing internal free Ca¹⁴. This provides a more striking way to demonstrate passage of Li and K through the conductance. In Fig. 3*a* and *b* the outer segment was initially exposed to a 'priming' solution containing 55 mM Na, 55 mM choline and 1 μ M Ca¹⁴, which caused the light-sensitive current to climb slowly to a high level. At the plateau level an additional 55 mM Na, Li or K was added and the resulting current increases were compared. From the slopes or initial peaks of the current increases, the Li:Na permeability ratio was found to be near unity and K:Na was about 0.7, both similar to the estimates obtained from 1 mM Ca experiments. The fact that the additional 55 mM Na caused rather little slow secondary increase in current after the initial jump also suggested that the conductance was near its maximum in the 55 mM Na, 1 μ M Ca solution.

In the same manner Rb and Cs were found to go through the light-sensitive conductance (data not shown). The permeability ratio Rb:Na was ~0.4–0.5, and Cs:Na was ~0.2–0.3. In addition, Rb had an inhibitory action like K. Since the relative selectivity for monovalent cations seems to stay unchanged in normal and low Ca conditions, low Ca perhaps simply increases the effective number or fractional open time of the conductance sites.

We have also found that the divalent cations Ba and Sr go through the light-sensitive conductance in both normal and low

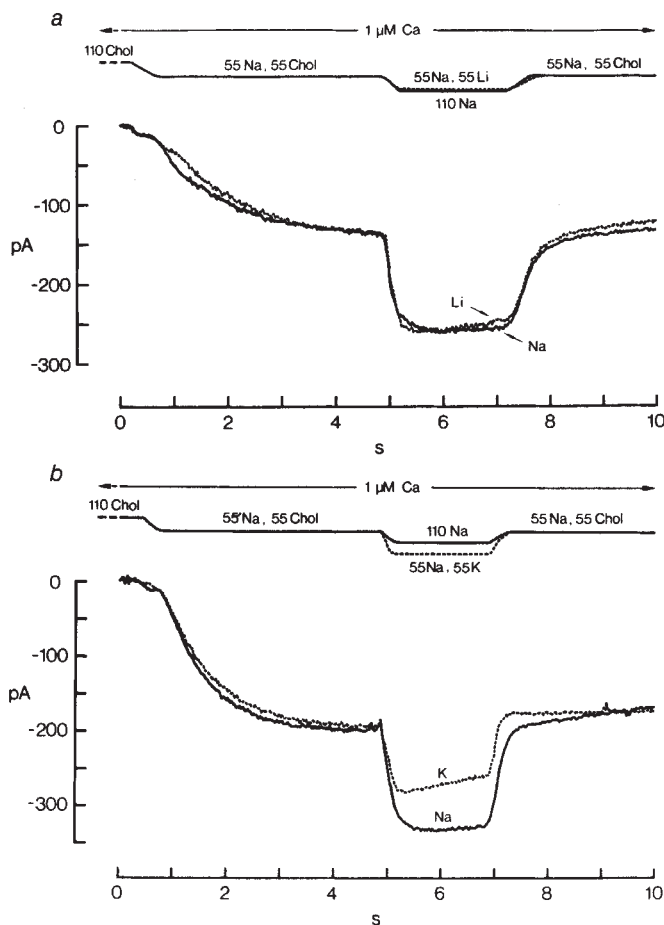


Fig. 3 Comparison of light-sensitive currents carried by Na, Li and K after the conductance was increased or 'primed' by a solution containing 55 mM Na, 55 mM choline and 1 μ M Ca. Two separate cells in *a* and *b*. Junction currents, shown magnified above the light-sensitive currents, indicate the time courses of solution changes. The junction currents are already subtracted in each trace. All traces are from single trials. In addition to Na, choline, Li and K in various isotonic substitutions, the buffered 1 μ M Ca solutions contained 2.5 mM K, 1.5 mM Mg, 0.093 mM Ca, 2 mM Mg-EDTA, 5 mM TMA-HEPES, 5 mM dextrose and were buffered to pH 7.6 (see ref. 14 for Ca buffering).

Ca solutions. A large light-sensitive Ca current was similarly detected when the rod outer segment was exposed to isotonic Ca immediately after the 55 mM Na, 1 μ M Ca priming solution mentioned above¹⁴, though for reasons still not well understood no Ca current could be observed when Na in normal Ringer's was completely replaced by Ca. Ba and Sr generally have permeation properties similar to Ca²⁰; it therefore seems most likely that Ca does go through the conductance in normal Ringer's. In physiological conditions such a Ca current is perhaps small relative to the Na current because of the large difference in their extracellular concentrations (1 mM versus 110 mM). A small but steady Ca influx through the light-sensitive conductance could, however, provide a simple replenishment mechanism for internal Ca, which otherwise would be gradually depleted by extrusion to the cell exterior in the presence of light^{9,10}.

The above experiments support and extend earlier observations that when external Ca is extremely low (≤ 1 μ M), cations other than Na can go through the light-sensitive conductance^{13,14,18,21,22}. They also underscore the key role of the Na–Ca exchanger in maintaining the open conductance and explain why earlier experiments with slower solution changes did not detect substantial ion movements besides Na in normal Ca conditions¹³. The relative abilities of Na and K to go through the conductance as measured above, together with estimates

of their internal concentrations in rods²³, are consistent with the reversal potential for the rod light response being at 0 to +10 mV¹⁵⁻¹⁷.

The rapid perfusion method was developed jointly with Professor Alan Hodgkin and Drs Peter McNaughton and Brian Nunn. They have independently obtained similar results on monovalent cations and have also provided comments on this paper. We also thank Drs Denis Baylor, Malcolm Brodwick, Lee Moore and John Russell for comments and discussions. The work was supported by US National Eye Institute and the Retina Research Foundation, Houston, Texas.

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Intracellular Na⁺ activates a K⁺ channel in mammalian cardiac cells

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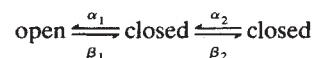
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In a wide variety of cells, various intracellular agents, such as Ca²⁺ (refs 1, 2), ATP^{3,4} and cyclic nucleotides^{5,6}, regulate ionic conductances of the membrane. In cardiac cells, the intracellular Na⁺ concentration ([Na⁺]_i) frequently increases when a disturbance occurs in the electrogenic Na–K pump activity or the Na–Ca exchange mechanism. We have investigated a possible role of [Na⁺]_i in controlling ion channels by using a patch-clamp method⁷, and have found a K⁺ channel that is gated by [Na⁺]_i > 20 mM, but not by the intracellular Ca²⁺ concentration (~10⁻⁴ M). We report here that the channel has a unitary conductance of 207 ± 19 pS (*n* = 16) with K⁺ concentrations of 150 mM outside and 49 mM inside, and shows no detectable voltage-dependent kinetics. The Na⁺-activated K⁺ channel represents a novel class of ionic channel.

In our experiments we used guinea pig ventricular single myocytes, dissociated by treatment with collagenase^{8,9} (0.04%; Sigma type I). In the inside-out patch clamp experiments, at least three types of single-channel activities were observed with a pipette solution containing 150 mM KCl. One type of channel was identified as the inward rectifier K⁺ channel^{10–12} as it had a slope conductance (γ) of 32 pS, a reversal potential (25–

30 mV) near the K⁺ equilibrium potential (E_K) of 29 mV, and a mean open time of 200–250 ms at –40 mV (Fig. 1ai). The opening probability of the channel decreased with hyperpolarization. For the second type of channel, γ was 94 pS (Fig. 1a_{ii}) and there was little voltage dependency in the opening probability. The reversal potential of this channel was 30 mV, which was also close to E_K . The channel opening was blocked by 2 mM ATP inside the membrane. Thus, this channel is identical to the ATP-sensitive K⁺ channel reported previously⁴. The third type of channel activity (Fig. 1a_{iii}), however, has not been described previously; the γ for this channel was 220 pS (range 179–240 pS) in the potential range –60 mV to 0 mV. The reversal potential was 28 mV (Fig. 1b; mean = 28.7 ± 5.5 mV, *n* = 12), which is very close to E_K . With 50 mM K⁺ and 100 mM Na⁺ in the pipette, γ was 77 pS (*n* = 3) and the reversal potential was 1 mV, suggesting a 58 mV change for a 10-fold change in external K⁺ concentration. Therefore, we conclude that this channel is selective for K⁺. The slope conductance decreased at potentials more positive than 20 mV with 150 mM K⁺ in the pipette (Fig. 1b), indicating an inward rectification of the single-channel current. Opening of the channel was usually accompanied by closures of short duration, which yielded bursts of openings (Fig. 1a_{iii}). With 100 mM Na⁺ on the inner side of the membrane, long intervals between bursts occurred only rarely. Therefore, the mean open and closed times were obtained from the arithmetical mean of the open or closed times. The mean open and closed times were 8.4 ± 2.0 ms and 6.5 ± 3.5 ms (*n* = 4), respectively, at 0 mV. The opening of the channel was unaffected by 2 mM ATP on the inner surface of the membrane. The probability of opening (*P*) was 0.49–0.54 and showed little voltage dependency at potentials between –40 mV and +20 mV (Fig. 1c). Thus, this type of K⁺ channel differs from both the inward rectifier channel and the ATP-sensitive K⁺ channel in single-channel conductance, mean open time and ATP sensitivity.

The value of *P* was found to depend on [Na⁺]_i. Figure 2a shows that the activity of the channel was almost negligible in Na⁺-free bathing solution (substituted by Tris⁺); activity appeared only after the bath was perfused with 50 mM Na⁺. We obtained similar results in eight other patches using Tris⁺ or choline⁺ as a Na⁺ substitute. In contrast, [Ca²⁺]_i of 10⁻⁴ M failed to activate the channel (Fig. 2b). In this patch, we observed two channels after activation with 100 mM Na⁺. Even when the channel was activated by Na⁺, increasing [Ca²⁺]_i to 10⁻⁵ M had no significant effect on *P*. The inability of Ca²⁺ to activate the channel was confirmed in three other patches. Figure 3 shows the dose–response relationship of [Na⁺]_i to *P* for five patches. The channel was activated at about 20 mM, and *P* was 0.55 ± 0.10 (*n* = 7) at 100 mM [Na⁺]_i. For a kinetic analysis, we considered, as a first approximation, a kinetic scheme having one open state and two closed states:



The transition rate constants (s⁻¹), calculated from the exponential distributions of the open and closed times¹⁰ for a particular case with 50 mM K⁺ in the pipette at –40 mV, were: α_1 = 422, β_1 = 398, α_2 = 14.2 and β_2 = 63.1 (t_o = 2.51 ms, t_f = 2.05 ms and t_s = 81.3 ms) with 30 mM Na⁺ inside, and α_1 = 518, β_1 = 235, α_2 = 44.3 and β_2 = 82.4 (t_o = 4.24 ms, t_f = 1.64 ms and t_s = 26.4 ms) with 50 mM Na⁺, where t_o , t_f and t_s are time constants of the open time distribution, and the fast and slow components of the closed time distribution, respectively. Thus, intracellular Na⁺ affects all the rate constants. Among these effects, the predominant one might be an increase of α_2 , which corresponds to a decrease in the interval between bursts. Within the pH range 8.0–6.5, *P* did not change significantly (*n* = 3). Li⁺ (100 mM) could not substitute for Na⁺ in the activation of the channel.

Further evidence for the activation of K⁺ conductance by [Na⁺]_i was obtained in whole-cell clamp experiments. In Fig. 4,

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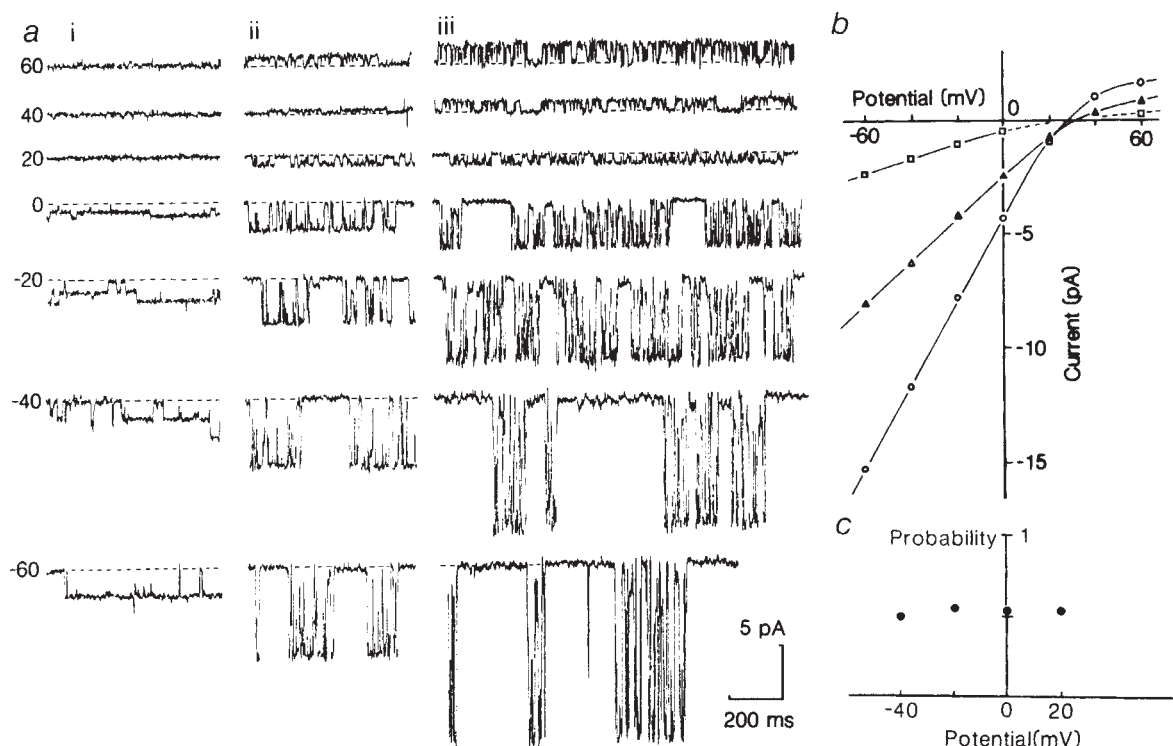


Fig. 1 Single-channel currents of three types of K⁺ channel in inside-out patches. *a*, Current traces (recorded at potentials between -60 mV +60 mV) of the inward rectifier channel (i), ATP-sensitive channel (ii) and Na⁺-activated K⁺ channel (iii). *b*, Current-voltage relationship of the channels in *ai* (□), *a ii* (Δ) and *a iii* (○). The conductances measured at the linear part of the inward current were 32, 94 and 220 pS, respectively. *c*, Opening probability of the Na⁺-activated channel in a different patch measured by integrating the current for at least 16 s. The compositions of the pipette and bath solutions were the same as for *a iii*. The pipette solution consisted of (in mM): KCl, 150; CaCl₂, 1.8; HEPES buffer, 5.0, pH 7.4. The bath solution for *ai*, *a iii* and *c* contained (in mM): KCl, 40; Na aspartate, 100; MgCl₂, 0.5; KH₂PO₄, 5.0; EGTA, 1.0; HEPES buffer, 5.0; glucose, 5.5; K₂-ATP, 2.0, with the pH adjusted to 7.4 by adding aspartic acid. In the patch illustrated in *a ii*, ATP was 0.5 mM; for the others the same concentration as in *ai* was used. Temperature of the perfusate was kept at 30–35 °C.

the inside of the cell was first dialysed with a Na⁺-free solution, while perfusing the bath with Ca²⁺-free Tyrode's solution. When the pipette solution was changed to 45 mM Na⁺ solution, there was a shift in the current-voltage curve consistent with the activation of an outward current. The reversal potential of the activated conductance was -66, -67, -73 and -75 mV in four trials with three cells, consistent with E_K of -74 mV. The current induced by intracellular Na⁺ shows an inward rectification around its reversal potential, but outwardly rectifies at potentials

more positive than -24 mV. It is not clear whether this is due to a property of the Na⁺-activated channel or an activation of other conductance systems.

These results indicate that intracellular Na⁺ regulates the gating of a specific type of K⁺ channel. The Na⁺-activated K⁺ channel can be characterized by its large conductance (207 pS), its inward rectifying property and its lack of appreciable voltage dependence of *P*. K⁺ channels of very high conductance (~240 pS) have been reported in several tissues^{1,3}, most of which

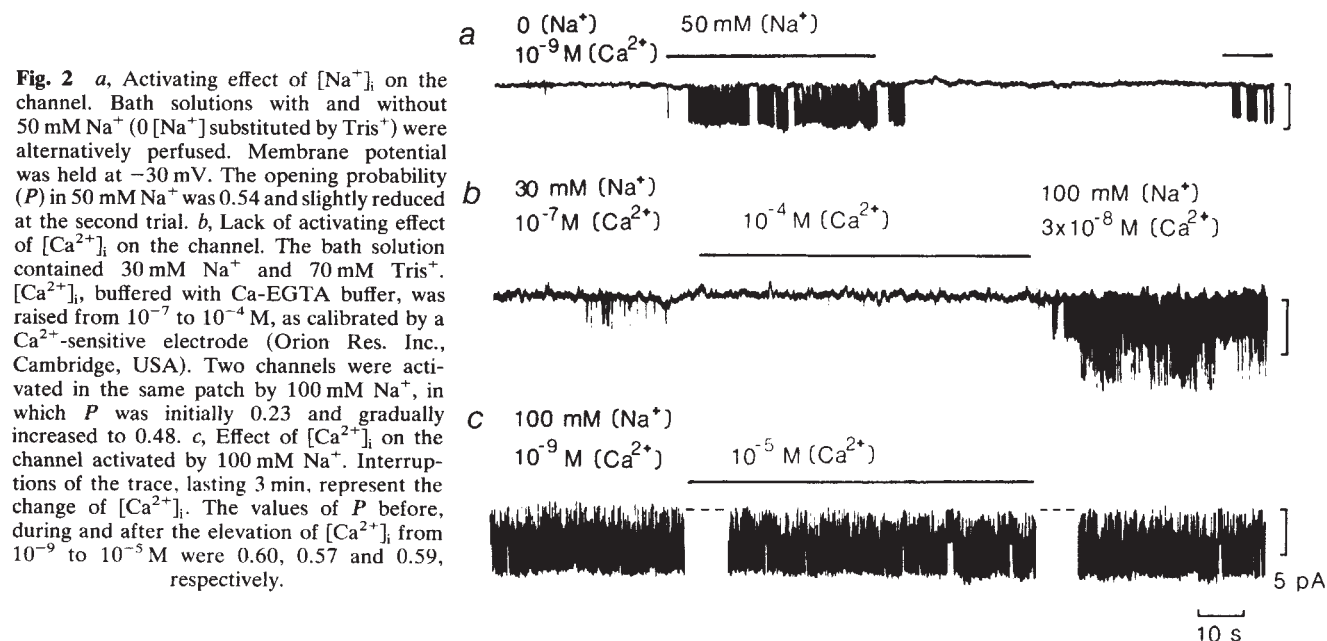


Fig. 2 *a*, Activating effect of [Na⁺]_i on the channel. Bath solutions with and without 50 mM Na⁺ (0 [Na⁺] substituted by Tris⁺) were alternatively perfused. Membrane potential was held at -30 mV. The opening probability (*P*) in 50 mM Na⁺ was 0.54 and slightly reduced at the second trial. *b*, Lack of activating effect of [Ca²⁺]_i on the channel. The bath solution contained 30 mM Na⁺ and 70 mM Tris⁺. [Ca²⁺]_i, buffered with Ca-EGTA buffer, was raised from 10⁻⁷ to 10⁻⁴ M, as calibrated by a Ca²⁺-sensitive electrode (Orion Res. Inc., Cambridge, USA). Two channels were activated in the same patch by 100 mM Na⁺, in which *P* was initially 0.23 and gradually increased to 0.48. *c*, Effect of [Ca²⁺]_i on the channel activated by 100 mM Na⁺. Interruptions of the trace, lasting 3 min, represent the change of [Ca²⁺]_i. The values of *P* before, during and after the elevation of [Ca²⁺]_i, from 10⁻⁹ to 10⁻⁵ M were 0.60, 0.57 and 0.59, respectively.

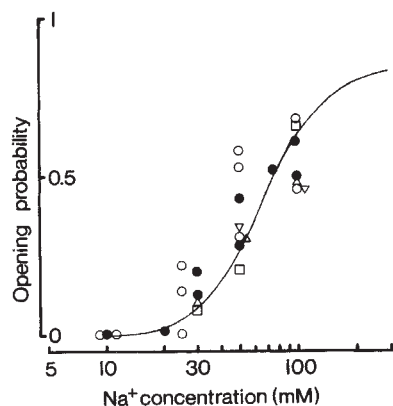


Fig. 3 Dose-response relationship between $[Na^+]_i$ and the opening probability of the channel. Measurements were performed in five different patches as indicated by the different symbols. A theoretical curve was drawn according to the following equation: $P = 0.85 / 1 + (K_D/[Na^+]_i)^n$, where K_D is a dissociation constant and n is Hill's coefficient. Using a least-squares fit, the values of K_D and n were found to be 66 mM and 2.8, respectively. The data indicated by solid circles were obtained in an opened cell-attached patch, where a part of the cell membrane was chemically skinned by treatment with saponin (10 mg ml^{-1}) while keeping the cell-attached patch intact.

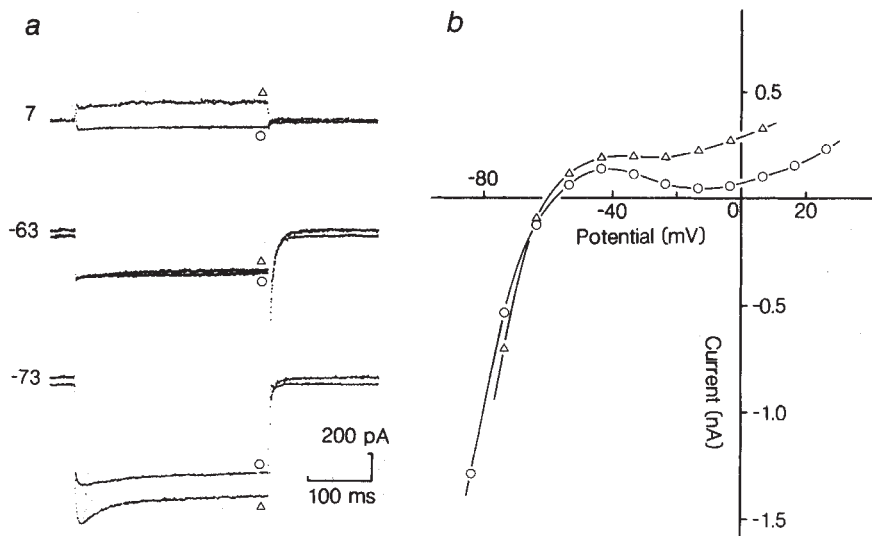


Fig. 4 Activation of a K^+ conductance by an increase in $[Na^+]_i$ in the whole-cell clamp. *a*, Current traces recorded with Na^+ -free internal solution (O) containing (in mM): K-aspartate, 90; Tris-HCl, 50; K_2 -ATP, 5; EGTA, 5, pH 7.4. Thereafter, 45 mM Tris-HCl was replaced with equimolar NaCl (Δ). The bath solution contained (in mM): NaCl, 143; KCl, 5.4; $MgCl_2$, 0.53; NaH_2PO_4 , 0.33; glucose, 5.5; HEPES buffer, 5.0, pH 7.4. The holding potential was set at -43 mV to inactivate the Na^+ current. Test potentials are indicated at the left of each trace. *b*, Current-voltage relationship for the cell in *a*. The slope conductance at -67 mV was increased by 36%.

are Ca^{2+} -activated K^+ channels. It is of interest that the inward rectifier current in starfish egg cells requires intracellular Na^+ to be activated by hyperpolarization¹⁴. A similar mechanism might underlie the interaction of Na^+ with the channel reported here.

In normal cardiac cells, $[Na^+]_i$ has been reported to be $<10 \text{ mM}$ (refs 15, 16), a concentration which is balanced between the influx through Na^+ channels, the Na-Ca exchange mechanism and other passive leakage pathways, and active extrusion via the Na-K pump¹⁷ as in other excitable cells. A prolonged failure of Na^+ pumping increases $[Na^+]_i$, resulting in a less efficient Ca^{2+} extrusion by the Na-Ca exchange, or even in a reversal of this mechanism due to a decrease in the Na^+ driving force^{18,19}. Activation of K^+ conductance by the elevated $[Na^+]_i$ might improve the Ca^{2+} transport by opposing depolarization of the cells.

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Adoptive transfer of myelin basic protein-sensitized T cells produces chronic relapsing demyelinating disease in mice

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The autoimmune disease of the central nervous system (CNS), experimental allergic encephalomyelitis (EAE), is induced by challenge of genetically susceptible animals with spinal cord homogenates or myelin basic protein (MBP)¹⁻⁴. Chronic and relapsing forms of the disease have some similarities to human demyelinating disorders, namely, multiple sclerosis^{3,5-7}, and are of particular interest. EAE can be transferred passively with sensitized lymphoid cells into syngeneic animals⁸ but transferred EAE has been believed to have limited relevance to human disease because it is usually monophasic and manifested by minimal demyelination⁹. We report here that a single transfer of MBP-sensitized lymph node cells or T cells, in the absence of a peripheral antigen depot, leads to both acute EAE with significant primary demyelination, and chronic relapsing disease with lesions typical of demyelination over a long period. These findings have major implications for the immunological mechanisms involved in experimental and human demyelinating diseases.

Guinea pig myelin basic protein (GPBP) was prepared by the method of Diebler *et al.*¹⁰. The purity of the preparation was established by polyacrylamide gel electrophoresis. Female SJL/J

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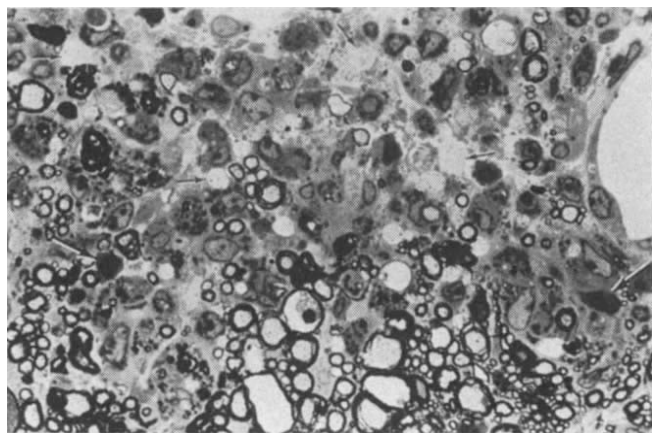


Fig. 1 Acute passively transferred EAE, 14 days post-transfer (1 μ m epoxy sections stained with toluidine blue). Thoracic spinal cord. Higher magnification shows demyelinated axons (small arrows), large mononuclear cells containing myelin debris and at the edge of the lesion, polymorphonuclear leukocytes (large arrows). Some axonal damage and unaffected fibres are seen below. $\times 600$.

mice, 6–10 weeks old, were injected subcutaneously at four sites, with 400 μ g GPBP and 30 μ g *Mycobacterium tuberculosis* in complete Freund's adjuvant¹¹. Ten days later, the animals were killed; the draining lymph nodes were removed and passed through mesh in Hank's balanced salt solution (HBSS) to obtain a single cell suspension. Total lymph node cell (LNC) suspensions or B-cell-depleted suspensions, prepared by panning on anti-mouse IgG coated plates¹², were adjusted to 4×10^6 per ml and cultured in the presence of GPBP¹¹. Four days later, the cells were removed and washed extensively. B-cell-depleted LNC were depleted further of adherent cells¹³ by two successive incubations on Petri dishes precoated with fetal calf serum for 90 min at 37 °C. The desired numbers of LNC or T cells in 0.2 ml were inoculated into the tail vein of syngeneic recipients.

Animals were observed daily for clinical signs of EAE. The severity of the disease was graded on a scale of 1–5 (ref. 11). Relapses were only scored when a mouse developed a similar or more severe neurological deficit following a previous improvement. Routine histology was performed as described

previously⁴. Selected animals between 14 days and 3 months after inoculation were perfused with 20 ml of 2.5% glutaraldehyde in PO₄ buffer and post-fixed in 1% osmium tetroxide. Blocks were dehydrated in ethyl alcohol and embedded in Epon 812. Epoxy sections 1 μ m thick were stained with toluidine blue¹⁴.

All recipients of $2-6 \times 10^7$ LNC or T cells developed clinical and histological signs of acute EAE 7–10 days after inoculation (Table 1, expts 1, 2). A few recipients of 7×10^6 cells showed a delayed onset of clinical disease as late as 82 days after transfer.

Approximately 20% of mice receiving 6×10^7 LNC died. After 2 weeks, the remaining mice with acute EAE either stabilized clinically, or gradually recovered with residual signs. At this time, some mice were killed for pathological examination and others were maintained for long-term clinical observations. Morphologically, the CNS of mice with acute disease showed perivascular inflammatory infiltrates in the meninges and parenchyma. The latter contained large lesions consisting of a primary wave of polymorphonuclear leukocytes which was followed by mononuclear cells. There was widespread loss of myelin but remarkable sparing of axons throughout the CNS, particularly in the subpial portion of spinal cord (Fig. 1); axonal degeneration was occasionally observed.

In five consecutive experiments, every mouse which recovered after the acute attack developed chronic relapsing disease. This usually began 30 days after the initial attack. After each relapse, the animals' conditions improved, but never returned to normal and with time there was progressive deterioration. Two representative experiments are shown in Table 1. In all studies, the potency of the adoptive transfer of chronic relapsing EAE, as judged by the number of relapses, the interval between relapses, clinical severity at the end of each attack and pathology was proportional to the number of LNC or T cells transferred.

Mice with chronic disease showed large lesions (Fig. 2) which were gliotic and contained inflammatory infiltrates consisting of a few polymorphonuclear leukocytes, many lymphocytes and macrophages. Although some axonal depletion was found, primary demyelination with sparing of axons was always evident and CNS remyelination was common (Fig. 3). The optic nerves were severely affected and dense collections of inflammatory cells were found within the angus of the lateral ventricles.

Chronic relapsing EAE has been induced in guinea pigs^{2,3} and mice⁴ by subcutaneous inoculation of CNS homogenates. Immunization with MBP may lead to relapsing disease in

Table 1 Adoptive transfer of chronic relapsing EAE in SJL/J mice by LNC primed *in vivo* and cultured *in vitro* with GPBP

Type and no. of cells transferred $\times 10^{-7}$	Day of first attack	Clinical stage at first attack	No. relapses and day after transfer each occurred	Clinical stages at each relapse
Expt 1*				
LNC				
6	6	3	5(42, 69, 119, 142, 168)	3, 4, 4, 5, 5
6	7	4	5(41, 72, 114, 139, 170)	3, 3, 4, 4, 5
6	7	4	4(39, 75, 118, 152b)	4, 3, 4, 5
6	6	4	1(38, died on 58)	
2	10	4	2(49, 95)†	4, 4
2	10	3	2(51, 92)†	3, 4
2	11	2	4(54, 84, 110, 159)	3, 3, 4, 4
2	11	2	3(51, 103, 153)	3, 4, 4
0.7	10	2	2(110, 159)	3, 3
0.7	10	2	2(116–165)	2, 3
Expt 2‡				
T cells				
2	10	3	3(27, 58, 89)	4, 4, 5
2	10	4	3(31, 60, 91)	2, 3, 5
2	10	4	3(35, 55, 88)	3, 4, 5
2	10	4	3(39, 58, 85)	1, 2, 3
0.7	10	2	2(64, 93)	1, 2
0.7	10	2	2(70, 95)	2, 2

* Observation period was 180 days for this experiment.

† Mice were killed for histological study.

‡ LNC were depleted of B cells before the culture and of macrophages after the 4-day culture. The observation period for this experiment was 95 days.



Fig. 2 Chronic relapsing EAE, 3 months post-transfer. Large, demyelinated gliotic lesions are seen in the dorsal and anterolateral columns (arrows). Upper cervical spinal cord. $\times 35$.

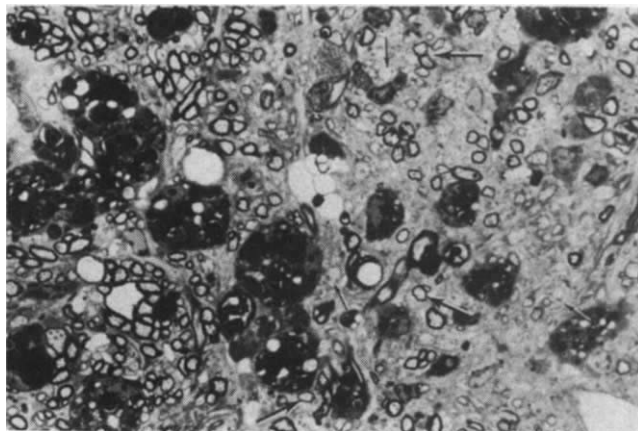


Fig. 3 Chronic relapsing EAE, 3 months post-transfer. At the edge of a chronic lesion, demyelinated axons (small arrows) and remyelinated axons (large arrows) can be seen. Towards the more normal white matter to the left, myelin degradation by large macrophages is apparent. $\times 600$.

rats^{15,16} and mice¹⁷. Injection of galactocerebroside with MBP augmented the level of demyelination observed in guinea pigs¹⁸, and contamination of MBP preparations with lipid components has been reported¹⁹. These observations raise the possibility that trace amounts of lipids in our antigen preparation could contribute to the recurrent disease. Although this cannot be excluded, we believe it is unlikely because MBP prepared by the method used in this study does not produce demyelinating disease in guinea pigs.

Only acute EAE has been transferred to naive recipients either by sensitized lymphoid cells^{8,11-16,20-23} or by T-cell lines²⁴. Previously it was shown that the cells responsible for the transfer of acute murine EAE are Lyt-1⁺2⁻ T cells¹¹. Recent work suggests that this subset of lymphocytes recognizes antigen in conjunction with class II molecules of the major histocompatibility complex (MHC). Although the reactive cells are directed at MBP, it is believed mostly to be localized in the major dense line of the myelin membrane²⁵ and therefore inaccessible to normal immune surveillance. Thus, reaction between MBP and immune cells may occur at some site other than the myelin membrane. MBP released during myelin turnover may be processed and presented to reactive cells. Indeed, macrophages²⁶, endothelial cells within the CNS²⁶ and oligodendroglia²⁷ have been reported to bear class II MHC molecules and could present antigen to immune cells. MBP is detectable in the blood of most species²⁸ which may help maintain immunological tolerance²⁹. If MBP enters the circulation via the vascular endothelial cells, the luminal surface of such cells may be a site for interaction between immune cells and MBP³⁰. This could lead to inflammation and result in subsequent myelin breakdown through a by-stander effect^{28,31}.

The mechanisms responsible for relapsing demyelinating disease in the absence of injected antigen are not known. One possibility is that MBP in antigen presenting cells is transferred along with the immune T cells and stimulates immune reactivity already present, but our previous findings with radiolabelled MBP indicate that the amount transferred would be less than 50 ng per animal¹¹. Further, T cells depleted of antigen presenting cells still produced the chronic disease (Table 1, expt 2). Another possibility is that the acute disease produced by transferred T lymphocytes causes release of myelin antigen(s). MBP released with other myelin components during tissue damage may be more immunogenic than soluble MBP, and lead to alternation between the diseased and tolerant states²⁹. Such antigen could either induce an immune response in the recipient

or lead to an expansion of transferred memory effector cells. Rats immunized with cells from effector T-cell lines obtained from MBP-primed animals developed acute EAE but recovered, although they continued to harbour anti-MBP memory T cells in the thymus for months after the cell transfer³². Finally it is also possible that the acute disease induces an immune response against components of myelin other than MBP. Identification of the pathogenetic mechanisms responsible for the chronic autoimmune disease are of considerable importance because similar processes could occur in human demyelinating disorders.

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Phagocytosing macrophages exclude proteins from the zones of contact with opsonized targets

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During receptor-mediated phagocytosis, macrophages release toxic molecules such as hydrogen peroxide which enable them¹ to kill antibody-coated tumour cells² and parasites³, too large to consume. Previous workers observed that while peroxide was clearly responsible for cytolysis of certain antibody-coated tumour cells^{2,4,5}, extracellular catalase was unable to inhibit this cytolysis, and they suggested that macrophages secrete peroxide into a protected cleft between the phagocyte and target. We have tested this and report here that the space beneath macrophages spread on glass surfaces is accessible to proteins with a molecular weight (MW) as large as 200,000 but the space beneath macrophages plated on glass surfaces coated with phagocytosis-promoting ligands is impermeable to proteins as small as 50,000 MW. It appears indeed that macrophages form a protein-tight seal at the periphery of their contact with ligand-coated surfaces and thereby create a closed compartment between the cell and the target.

To examine the ability of soluble proteins to permeate the zone of contact between a macrophage and a ligand-coated target, glass slides were coated with dinitrophenol (DNP)⁶ and then with a monoclonal IgM anti-DNP antibody to give a IgM-coated surface (Fig. 1). IgM is not recognized by receptors on the macrophage, and therefore they spread on these surfaces as they would on glass or plastic. The purpose of the IgM-coated surfaces to provide binding sites for fluoresceinated anti-IgM antibodies. In parallel experiments, we incubated the DNP surfaces with a mixture of IgG-anti-DNP and IgM-anti-DNP (Fig. 1). The Fc domain of IgG is recognized by Fc receptors on macrophages, thus when the cells settle on such IgG-bearing surfaces they produce hydrogen peroxide¹ and spread extensively as though in an attempt to phagocytose the slide.

Human monocytes cultured for 4–12 days in Teflon beakers⁷ were allowed to adhere to one of these ligand-coated surfaces. They were then incubated at 20 °C for 1.5 h with fluoresceinated anti-IgM, washed, fixed, and viewed with a fluorescence microscope. This antibody binds uniformly to IgM-coated substrates, even directly under the macrophage (Fig. 2c) and indicates that the space between the undersurface of adherent macrophages

and the substrate is accessible to soluble proteins. In contrast, on IgG + IgM-coated surfaces, the fluoresceinated anti-IgM is excluded from the zone between the phagocyte and the substrate leaving 'black holes' in the fluorescent surface that correspond closely to the margins of the spread macrophage (Fig. 2a). This suggests that during phagocytosis, macrophages make a tight seal against the target surface that excludes soluble protein.

We have done several experiments to eliminate alternative explanations of these observations. The exclusion of fluoresceinated anti-IgM from the space beneath a phagocyte on an IgG-coated surface is not a consequence of the greater spreading or the evolution of peroxide seen when macrophages interact with IgG. If cells are treated with the tumour promoter, phorbol myristate acetate (PMA), the cells spread extensively and evolve large amounts of peroxide^{1,8}. However, PMA-treated cells do not exclude fluoresceinated anti-IgM from their regions of contact with control IgM-coated surfaces (data not shown). Further, neither phagocytes nor their secretion products quench the antibody fluorescence because, when fluoresceinated anti-IgM is added to the IgG + IgM-coated surface before the cells are plated, the surface remains uniformly fluorescent. Finally, the cells do not consume or destroy the antigens recognized by the fluorescent antibody. When cells that had formed black holes are permeabilized with 1% Triton X-100 (in phosphate-buffered saline, 10 min, 5 °C), the IgM underlying the former holes can be stained with fluorescent antibody (data not shown).

We have confirmed our observations using electron microscopy. For these studies, we used horseradish peroxidase-labelled IgG anti-IgM (HRP anti-IgM) instead of fluorescent antibody, and the HRP anti-IgM was visualized by staining with diaminobenzidine. Sections cut perpendicular to the substrate show that HRP anti-IgM readily penetrates beneath macrophages spread on IgM-coated surfaces (Fig. 3a), but HRP anti-IgM cannot penetrate beneath macrophages engaged in a 'phagocytic' interaction with IgG + IgM-coated surfaces (Fig. 3b). The penetration of HRP anti-IgM stops within 0.28 (± 0.15) μm of the margin of the cell, in a zone where the plasma membrane appears tightly adherent to the substrate. The entire plasma membrane is not, however, tightly adhered to IgG + IgM-coated surfaces. The membrane underlying the central area of the macrophage resembles that of a macrophage spread on a control substrate with interspersed regions of close apposition and regions well separated from the substrate (Fig. 3). Thus, there exists an open space or compartment between the cell membrane and substrate that is inaccessible to external proteins.

The exclusion of protein from the macrophage-substrate interface is not a simple physical consequence of receptor-ligand bridging. Macrophages have receptors for the third component of complement, C3, that mediate binding but not phagocytosis

Fig. 1 Construction of ligand-coated surfaces. Masked slides (Carlson Scientific) were treated with poly-L-lysine then with 2,4-dinitrobenzene sulphonate to yield DNP-coated surfaces⁶. These surfaces were treated with 10 μl of murine monoclonal IgM anti-DNP (1:200 dilution of ascites fluid) with or without 10 $\mu\text{g ml}^{-1}$ of affinity purified rabbit IgG anti-DNP (ref. 15) for 30 min at 20 °C. The surface was washed and macrophages were allowed to adhere to it. Fluoresceinated goat anti-IgM antibodies (IgG or the F(ab')₂, or Fab fragments (Cappel)) were diluted 1:100 in phosphate-buffered saline and added for 1.5 h at 20 °C. Fluoresceinated F(ab')₂ fragments are depicted here.

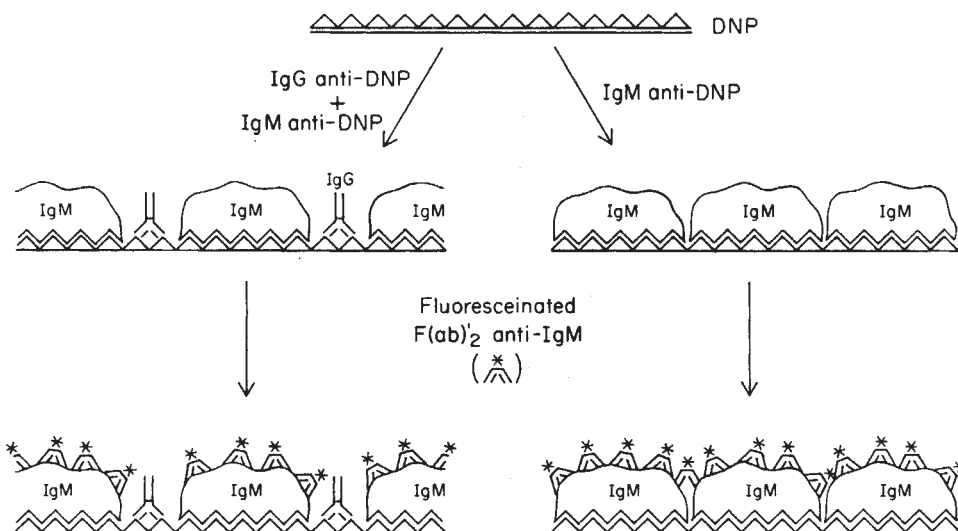


Fig. 2 Staining of ligand-coated substrates with fluoresceinated anti-IgM. Macrophages were cultured for 10 days in Teflon beakers, collected, then allowed to spread for 45 min at 37 °C on IgG + IgM-coated surfaces (*a, b*) or IgM-coated surfaces (*c, d*). The surfaces were then incubated for 1.5 h at 20 °C with a 1:100 dilution of fluoresceinated anti-IgM. Preparations were then washed, fixed in 3% formalin (diluted in phosphate-buffered saline) for 30 min at 20 °C, and photographed with a fluorescence microscope. The right-hand panels (*b, d*) are phase contrast photographs, and the left-hand panels (*a, c*) are fluorescent photographs of the same fields. Note that the IgM-coated surface underlying the cells is uniformly stained with the fluorescent antibody (*c*) but that cells spread on the IgG + IgM-coated surface protect the underlying antigens from binding the fluorescent anti-IgM (*a*). The centre of the cell appears bright owing to yellow autofluorescence that is easily distinguished from the green fluorescence of the antibody. In this experiment, fluoresceinated F(ab')₂ fragments of anti-IgM were used. Similar results were obtained using fluoresceinated Fab fragments or fluoresceinated whole IgG. Scale bar, 20 µm.

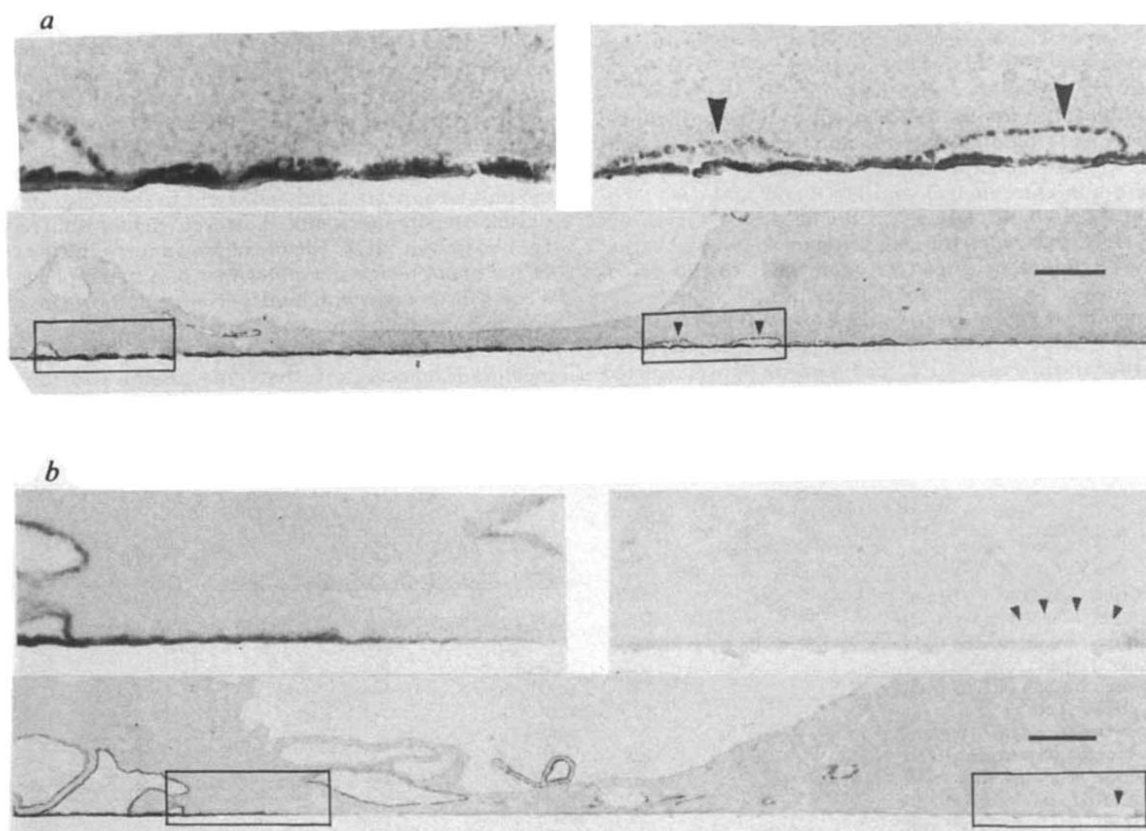
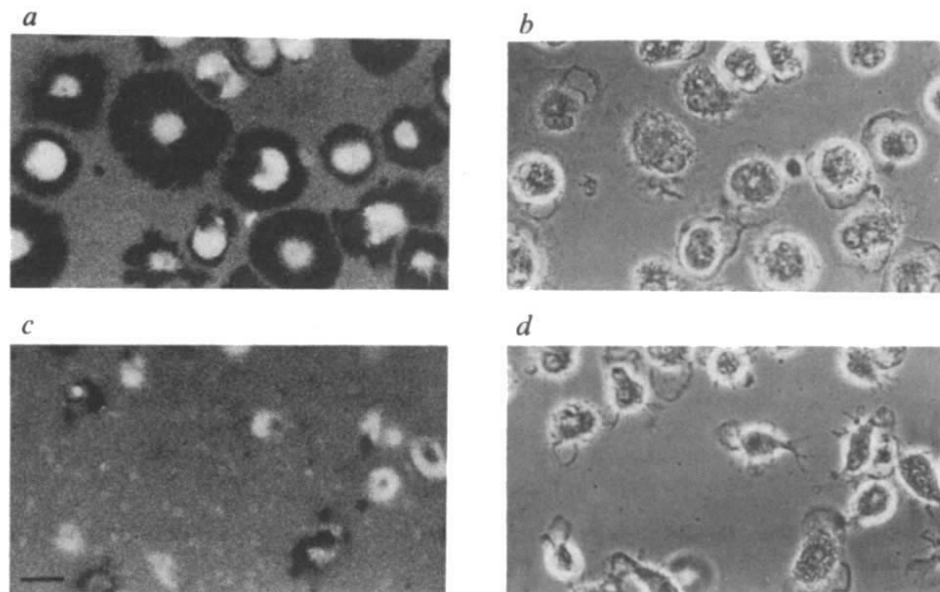


Fig. 3 Ultrastructural localization of horse radish peroxidase-labelled anti-IgM (HRP anti-IgM) on ligand-coated substrates. Macrophages were cultured for 8 days in Teflon beakers, collected, then allowed to spread for 45 min at 37 °C on IgM-coated surfaces (*a*) or IgG + IgM-coated surfaces (*b*). The surfaces were then incubated for 1.5 h at 20 °C with a 1:100 dilution of HRP anti-IgM, washed, then fixed for 1 h at 20 °C with 2.5% glutaraldehyde. HRP was stained using diaminobenzidine¹⁶, the specimens were treated with 1% osmium tetroxide, and were then embedded in resin¹⁷. Sections cut perpendicular to the surface were viewed in an electron microscope without further staining. The electron-opaque reaction product generated by HRP is seen coating the entire IgM-coated surface, even directly under spread macrophages (*a*). In contrast, reaction product is not seen beneath cells spread on IgG + IgM-coated surfaces (*b*), even in locations where the plasma membrane rises from the substrate revealing an open space between the membrane and substrate (arrows). Boxed areas are shown in 3.5-fold enlargements. Scale bars, 0.1 µm.

of C3-coated erythrocytes⁷. We therefore constructed surfaces bearing C3 by incubating IgM-coated surfaces with a source of complement⁹. When macrophages spread on such surfaces C3 receptor activity is lost from the apical portion of the cell^{7,10} because of diffusion of the receptors to the basal surface where they are 'trapped' by interaction with ligand¹¹. However, despite this ligand-receptor interaction IgG anti-C3 can still penetrate readily beneath the macrophage. (To observe this, macrophages on C3-coated surfaces were cooled to 20 °C, then incubated for 45 min with murine IgG anti-C3, then for another 45 min with fluoresceinated Fab anti-IgG and examined by fluorescence microscopy.) In contrast, stimulated macrophages are able to phagocytose via their C3 receptors; the addition of 30 ng ml⁻¹ PMA to macrophages cultures increases phagocytosis promoted by C3 receptors 10–50 fold⁷. Macrophages plated on a C3-coated surface in the presence of PMA exclude IgG anti-C3 from the interface with the substrate (data not shown). These experiments demonstrate that sealing of the contact zone between macrophage and target is not caused by receptor–ligand interaction alone but that cytoplasmic movements initiated by receptor–ligand interaction causes the sealing.

The spreading of cultured cells on glass or plastic has been likened to the cells' attempt to phagocytose a particle of infinite diameter. Here we show, however, that spreading and phagocytosis appear quite different. After spreading, the space between macrophage and substrate is freely accessible to proteins as large as 200,000 MW, but during a 'phagocytic' interaction with the substrate, proteins as small as 50,000 MW (Fab fragments of fluoresceinated anti-IgM) are excluded from the phagocyte–substrate interface. Thus it appears that the seal between macrophage and substrate is brought about by cellular movements characteristic of phagocytosis but not by the movements of spreading.

During phagocytosis, macrophages form the seal as a continuous rim at the leading edge of the advancing pseudopod, thus creating a closed compartment between phagocyte and target (Fig. 3). This observation is consistent with previous electron microscope studies which show the phagocyte's plasma membrane tightly appressed to the target at the leading edge and an open space between the macrophage and target at the base of the forming phagosome^{11,12}. The presence of such a protected compartment has been predicted from experiments on the cytolytic action of phagocytes^{2,4,5} and from studies of ligand-induced modulation of Fc receptors^{13,14} and the data presented here provide evidence for its existence. We believe that this compartment is used to concentrate, protect and target toxic substances that may be specifically released into this space by the phagocyte.

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Separation of developmental compartments by a cell type with reduced junctional permeability

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Two recent reports^{1,2} have suggested that the junctional membrane of the cells located at compartment boundaries in developing insect epithelia, although permeable to inorganic ions^{3,4}, prevents the cell-to-cell transfer of the fluorescent tracer Lucifer yellow (LY). However, cells within a compartment are linked by junctions permeable to both inorganic ions and organic tracers^{1–5}. These findings imply that junctional communication is important in tissue development and that spatial selectivity may allow developmental autonomy in nearby compartments. The observation that LY was occasionally seen to pass across the segment border of the milkweed bug *Oncopeltus fasciatus*¹ suggested to us that the permeability of the junctions of the segment border may be locally controlled. We report here that the selectivity in the junctional channels at compartment boundaries in this insect is variable and under the control of a discrete population of border cells. The extent to which these cells reduce the rate of tracer passage across the compartment boundary relative to that within the compartment is influenced by the culture medium in which the tissue is placed.

Pieces of integument, containing three segment borders, were dissected from the dorsal abdominal wall of fourth instar nymphs of the mutant strain *cb;wb* (ref. 6). Epidermis of this stage was selected over that of the fifth instar studied previously¹ because the cuticle is smoother at the segment borders and allows greater cellular detail to be seen. The preparation was clamped in a culture dish containing one of three bathing media (Table 1), and examined at ×40 objective magnification by phase-epifluorescence⁷. Routine methods were used to micro-inject dye into cells by iontophoresis⁸.

The transverse border of each segment is usually marked by the presence of several rows of elongated cells ('border cells') with their long axes parallel to the border⁹. The pigmentation and clonal origin of these cells suggest that they have a common lineage with the more posterior segment, that is, they are at the leading edge of the segment⁹. The border cells confront the most posterior cells of the segment ahead. Both these posterior cells and the general cells of the posterior segment are normal in shape (regular polygons) (Figs 1–3).

Three fluorescent dyes were used to probe the junctional permeability of the border cells: Lucifer yellow (LY, molecular weight 457), 6-carboxyfluorescein (CF, molecular weight 376), and lissamine rhodamine B (LRB, molecular weight 559). Dye was injected into a normally shaped cell one or two cells distant from the strip of border cells. The extent of dye spread was recorded photographically (Figs 1, 2), while the rate of dye passage in the cell sheet was determined from spreads videotaped during dye injection^{10,11}.

The cell-to-cell spread of all three dyes was radially asymmetrical (Fig. 1), as their passage into and across the strip of border cells was always retarded. In a typical LY spread (Fig. 1a), appreciable amounts of the dye passed into the cells next to the source cell within 3 s, and into the border cells within 10 s. A drop in fluorescence intensity coincided with the margins of the border cells. Within 30 s the dye had passed across the border into the normally shaped cells of the next segment, to image cell detail such as nuclei, vacuoles and cell margins. A dead cell in the recipient segment was silhouetted by the dye diffusing around it. This was observed irrespective of whether the source cell was anterior or posterior to the border. The 'texture' to the dye spread is proof that the dye passed from cell to cell and was not due to scattered light or to the dye passing between the cells and the underlying cuticle. The general

Fig. 1 Fluorescent tracers microinjected into epidermal cells near the segment border pass across it, although a drop in fluorescence occurs in this region. Left: record of three different fluorescent tracers spreading rapidly across the segment border. Right: the same preparations shown in phase contrast. The position of the border is marked by the arrowheads above and beneath the micrographs. A, anterior segment; P, posterior segment. Scale bar, 25 μ m.

a, Lucifer yellow (LY) was injected into a cell (+) anterior to the segment border for 30 s, the electrode was then removed and the extent of dye spread photographed immediately (60 s exposure). The spread of dye is impeded by the border. The appearance of the cells, imaged by dye localized in their nuclear regions, suggests that the border cells were not particularly elongated in this preparation. The source cell was damaged during electrode removal and failed to retain the dye. The dark area just across the border from the injection site is a dead cell imaged by the dye that diffused around it. **b**, Phase-contrast appearance of **a**. The most posterior cells of one segment are more opaque than the anterior cells of the adjacent segment. In some cells of preparations such as this one, the presence of small vesicles that exclude dye (diagonal arrow in **a** and **b**) allow the micrograph pairs to be matched up independently of the cell margins, which are often obscure. **c**, Carboxyfluorescein (CF) injected for 20 s, into a cell (+) posterior to the segment border, and the extent of its spread photographed (7 s exposure). CF has crossed the border within 30 s of the start of injection. This dye fills the individual cells more uniformly than does LY (**a**). The tip of the electrode (**e**) was left in the source cell after the period of dye injection was over. **d**, Phase-contrast appearance of **c**. In the centre of the micrograph the position of the segment boundary is marked by a thin intercellular space (horizontal arrow). Cell margins, although generally difficult to discern here, are seen as step-like drops in fluorescence in **c**. **e**, Lissamine rhodamine B (LRB) injected continuously for 120 s into a cell (+) in the anterior compartment and the spread photographed (12 s exposure) with the electrode (**e**) still inside the source cell. This dye was the slowest to image cell detail across the border. **f**, Phase-contrast appearance of **e**.

Methods: Cell preparations were excised from fourth instar nymphs in the middle of the instar, placed in medium 3 (ref. 15) and used within 60 min of dissection. Dye was iontophoresed from an electrode (200-ms current pulses, 1 per s; strength in **a**, 60 nA; in **c** and **e**, 6 nA) inserted into a normally shaped cell (the source cell) one or two cells distant from the border cell strip. Placing the electrode in this position prevented the tip from crossing the border accidentally, either during its insertion into the source cell or due to any slight electrode drift during injection. Both could give a false positive for the dye passage across the border. The source cell was close enough to the border to allow dye to bank up against it, and passage across it to be detected. The entry of the dye electrode into the source cell was timed from the moment its membrane potential was recorded on the chart recorder. Criteria for a successful dye spread were a stable membrane potential, between 26 and 35 mV during the period of dye injection, and the presence of cell detail in the fluorescence image.

features of the cell-to-cell passage of CF and LRB both within and between segments were the same as those for LY, although the rates differed. The spread of LRB was considerably slower than that of the other two dyes, as has been noted in another insect epidermis¹¹.

We noticed also that the rate of dye spread was considerably influenced by the medium in which the tissue was placed (Table 1). In some media, such as that used for the preparations shown in Fig. 1, the border offers little resistance to dye spread; in others, dye passage is almost totally blocked in the border region. This observation suggests that the cells at the segment border can regulate the segment-to-segment passage of cytoplasmic molecules, but does not indicate whether the asymmetrical spread of dye is the result of tighter packing of border cells

(since dye spreads more rapidly within cells than between cells) or whether the permeability of the junctional channels in the border region is set at a different level from that elsewhere in the segment. To show that the altered shape of these cells is not the sole cause of the impeded dye spread, some preparations were placed in medium that depressed segment-to-segment dye coupling, a cell posterior to the border cells was injected with CF, and the extent of its spread photographed. The procedure was repeated on a cell anterior to the border, directly opposite the first cell. The result (Fig. 2) has two main features. First, dye spread is virtually blocked in the border region in both cases (in contrast to Fig. 1). It is unlikely that this could be due to changes in cell packing. Second, the lines along which the dye movement is restricted are not the same, but coincide instead

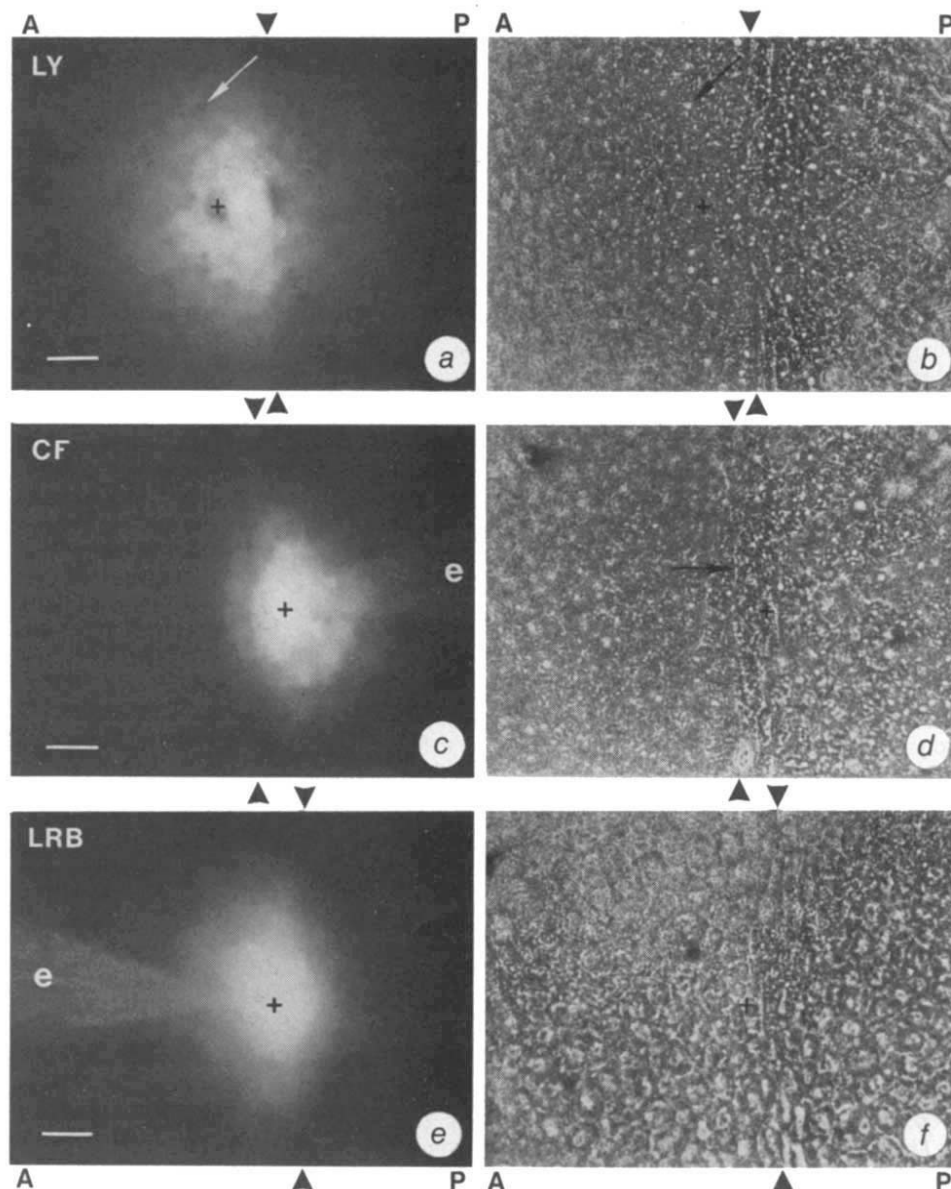
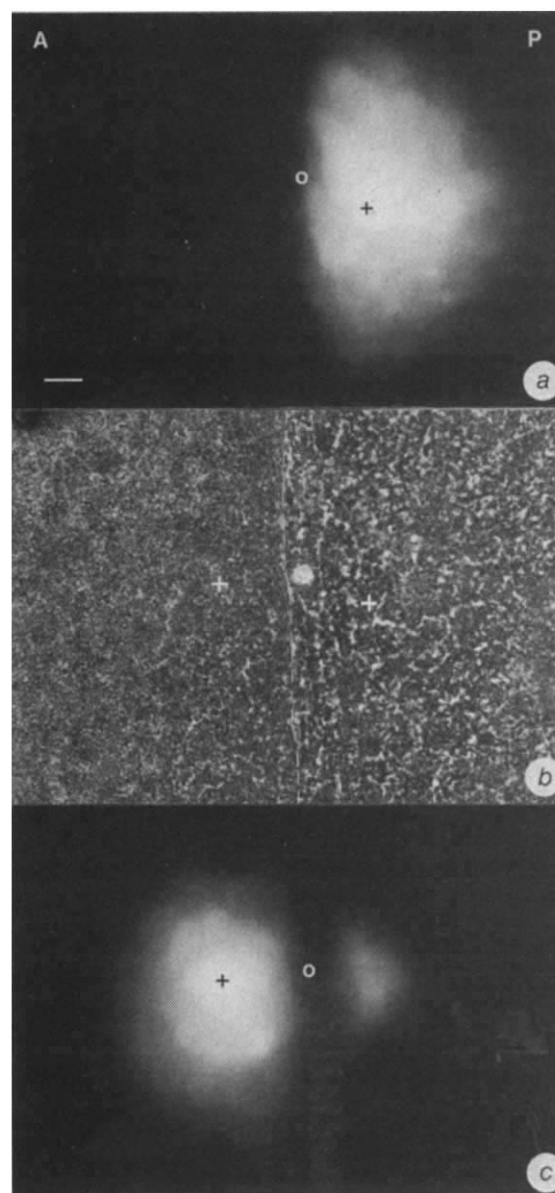


Fig. 2 When tracer was injected into a cell near the segment border in preparations immersed in medium 1 of Table 1, its spread was blocked along a line parallel to the segment border. Dye injected into the anterior segment was blocked at the border while that injected posterior to it was blocked at a line that did not coincide with the segment border, but was separated from it by a strip of cells. The junctional permeability of this border strip of cells appears to be regulated independently, since dye moved rapidly among cells within a segment. CF was injected (200-ms pulses of 60 nA, 1 per s) into cells labelled +. A, anterior segment; P, posterior segment. Scale bars, 10 μ m. **a**, CF injected into a cell posterior to the segment border for 9 s and the extent of dye spread photographed 22 s later (4 s exposure) with the electrode removed. The dye spread in a radially asymmetrical fashion and was blocked in its passage along a line passing through a marker particle (white circle, see **b**). Contrast the shape of this spread with that in Fig. 1c, where the spread of dye is only slightly retarded in the border region. **b**, Phase-contrast view of the cells shown in **a** and **c**. The more opaque cells of the anterior segment are distinguishable from the clearer cells of the posterior segment; the position of the segment border is emphasized by the dashed white line. The refractile particle lying just to the right of the segment border serves as a reference for locating it in **a** and **c**. **c**, Dye injected for 8 s and its fluorescence photographed 23 s later (4 s exposure) with the electrode removed. Dye spread is asymmetrical with its movement strongly truncated at the segment border, just to the left of the marker particle (white circle). Residual fluorescence from the spread shown in **a** done 4.5 min earlier is seen to the right of the marker particle. A drop in tracer fluorescence at the border could be an artefact caused by a reduction in cell thickness or changes in the cuticle in this region. Histological sections, however, showed the integument to be flat and the cell and cuticle thickness to remain constant across the border in our fourth instar preparations. Since the fluorescence was viewed through the cuticle, it is also possible that a reduction in the light-transmitting properties of the cuticle might lower fluorescence at the border. Consequently, preparations injected with dye were then inverted and re-examined. With the cuticle removed from the light path, no change in the intensity and distribution of fluorescence was seen.



with the margin of the border cell population nearest the injection cell. In both cases, the dye spread one or two cell diameters towards the border before being stopped by the border cells, while at the same time spreading four to five cell diameters away from the border. Here, it appears that there is a separate population of border cells having greatly reduced junctional permeability.

The presence of a cell type with different junctional properties can also be demonstrated by injecting dye simultaneously into two cells, one on either side of the border (Fig. 3). A narrow strip of elongated cells, lying just posterior to the segment border, is revealed by its lower tracer permeability, despite a high concentration of dye potentially able to enter these cells from both sides. When a border cell identified in this way was subsequently injected with dye, it passed at best slowly and radially into the surrounding cells. No preferential movement of dye among the border cells was detected.

Although Warner and Lawrence¹ clearly demonstrated an alteration in the normal cell-to-cell diffusion of microinjected tracers at the compartment border, they did not explain how this was achieved. The data suggested, nevertheless, that reduced junctional permeability at the border results from the confrontation of the surfaces of cells belonging to different compartments. This implies that communication is reduced only at areas of membrane junctions connecting cells of adjacent compartments, but that it remains normal at junctions connecting border cells with other cells in the same compartment. However, this appears not to be the case. *In vitro*, reduced communication between compartments in the bug is due to the intervention of a discrete population of cells (located at the anterior margin of each segment) whose junctions in certain culture media all have reduced permeability. These cells communicate less not only with the cells of the compartment anterior to them, but also among themselves and with the general epidermis of their own

Table 1 Extent of cell-to-cell spread of three fluorescent tracers across the segment border

Tracer	No. of preparations tested	Appearance of individual dye spreads		
		a	b	c
Carboxyfluorescein	29	13 (1)	69 (1-3)	3 (3)
Lucifer yellow	10	0	25 (1-3)	8 (2, 3)
Lissamine rhodamine B	19	10 (1)	36 (1-3)	2 (3)

About equal numbers of spreads were prepared in which the source cell lay anterior or posterior to the border. No differences in dye spread were noted. **a**, Dye spread restricted to within one segment by border cells; **b**, dye spread into adjacent segment but impeded by border cells; **c**, dye spread minimally retarded by border cells. As we were concerned that the composition of the culture medium might influence junctional permeability, three media were tested. Each preparation was used in only one medium. Numbers in parentheses refer to the media in which a particular result was obtained. Medium 1 was TC 199 containing 10 mM PIPES buffer, medium 2 was leafhopper medium¹⁴ and medium 3 was a *Drosophila* medium¹⁵ lacking amino acids. The media contained 10% fetal calf serum and the pH was adjusted to 6.7 with NaOH. Of the three, medium 3 least retarded the movement of fluorescent tracer across the border. It also resembled most closely the blood of phytophagous hemiptera in its monovalent and divalent cation levels.

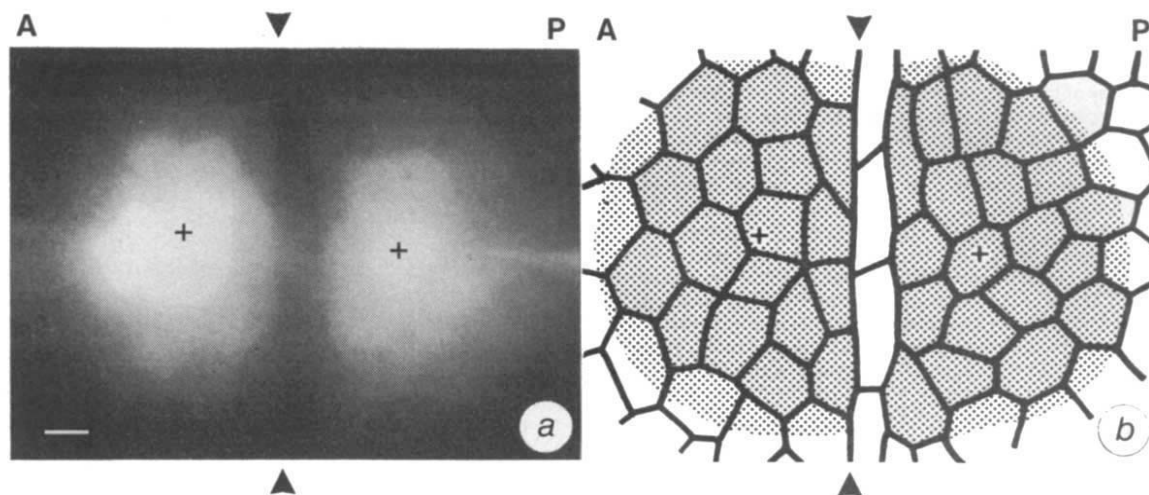


Fig. 3 The cell-to-cell spread of LRB injected into a cell on each side of the border simultaneously outlines a border strip of cells slow to receive the dye. The reduced permeability of these cells may explain how molecular interactions between compartments are limited. Arrowheads mark the position of the segment border. A, anterior segment; P, posterior segment. Scale bar, 10 μ m. LRB was injected into the two cells (+) with 200-ms current pulses of 5 nA, 1 pulse per s. In **a**, the extent of spread of LRB was photographed between 20 and 30 s after injection (exposure time 10 s). In this preparation the border strip of cells only partially restricted the segment-to-segment spread of dye. While dye spread extensively among the cells of each segment, it was retarded sufficiently in the border region to outline the border cells. **b**, Margins of the cells seen in **a** drawn from their combined fluorescence and phase-contrast images, showing the extent of the dye spread (stippled areas) from the two injection sites. As in Fig. 2, the border strip (not stippled) appears to consist of a single row of cells.

compartment (similar to a mechanism that may exist at compartment boundaries in the *Drosophila* wing disc²). The ability of the border cell junctions to block selectively the passage of organic tracers in certain conditions *in vitro*^{1,2} suggests that these cells may modulate the size range of molecules able to pass from segment to segment at different times in development.

Other distinct behaviours of the border cells include their rapid migration and proliferation during wound healing^{12,13}.

Their reduced junctional communication and characteristic behaviour may allow these cells to remain neutral in their gradient properties¹² and hence serve to establish and maintain discontinuity between the segmental compartments.

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Expression of a $V_H C_\kappa$ chimaeric protein in mouse myeloma cells

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The heavy (H) and light (L) chains of antibodies consist of variable (V) and constant (C) regions. The V regions of the heavy and light chains form the antibody combining site^{1,2}. To determine whether a V region could be functional when joined to a polypeptide other than its own C region, we constructed a chimaeric gene encoding the V region of a mouse heavy chain and the C region of a mouse κ light chain ($V_H C_\kappa$). The heavy-chain gene is derived from an A/J mouse hybridoma cell line 36-65 whose antibody product ($\gamma 1, \kappa$) is specific for the hapten azophenylarsonate³. We report here that, when introduced into a mouse myeloma cell line, the

chimaeric gene is expressed and a protein of the expected molecular weight is secreted into the medium. As light chains tend to dimerize^{4,5} we expected that the $V_H C_\kappa$ protein might associate with light chain from the cell line 36-65 to form an antibody-binding molecule. Affinity binding experiments and K_a determination indicate that this is the case. Dimers of this type offer a novel and interesting alternative to existing antibody-binding molecules.

Figure 1 shows the scheme used to construct two plasmids, $\Delta H p V$ and $\Delta H p V E$, bearing the $V_H C_\kappa$ chimaeric gene and the selectable marker *Ecogpt*^{6,7}. Both $\Delta H p V$ and $\Delta H p V E$ contain the rearranged 36-65 V_H gene with its 5' flanking sequence⁸, and DNA encoding the C region of the κ light chain of the S107 BALB/c mouse myeloma cell line⁹. A chimaeric intron separates the V_H and C_κ genes; this intron includes the light chain transcriptional enhancer sequence (E_κ)^{10,11} (V.T.O. and S.L.M., unpublished results) in $\Delta H p V$, and both E_κ and E_H , the heavy chain transcriptional enhancer sequence^{12–14}, in $\Delta H p V E$ (Fig. 1).

$\Delta H p V$ and $\Delta H p V E$ were separately transfected into J558L BALB/c mouse myeloma cells by protoplast fusion¹⁵ as described elsewhere¹⁶. (J558L is a variant of the J558 cell line¹⁷ that synthesizes and secretes only a λ light chain but no heavy chain.) Transformants containing the *Ecogpt* gene were selected in medium containing mycophenolic acid^{6,7}. Ten transformants from each of the two transfection experiments were analysed for production of $V_H C_\kappa$ by immunoprecipitation with rabbit antiserum specific for the C region of mouse κ chain, and separately with rabbit anti-idiotypic (Id) antiserum specific for

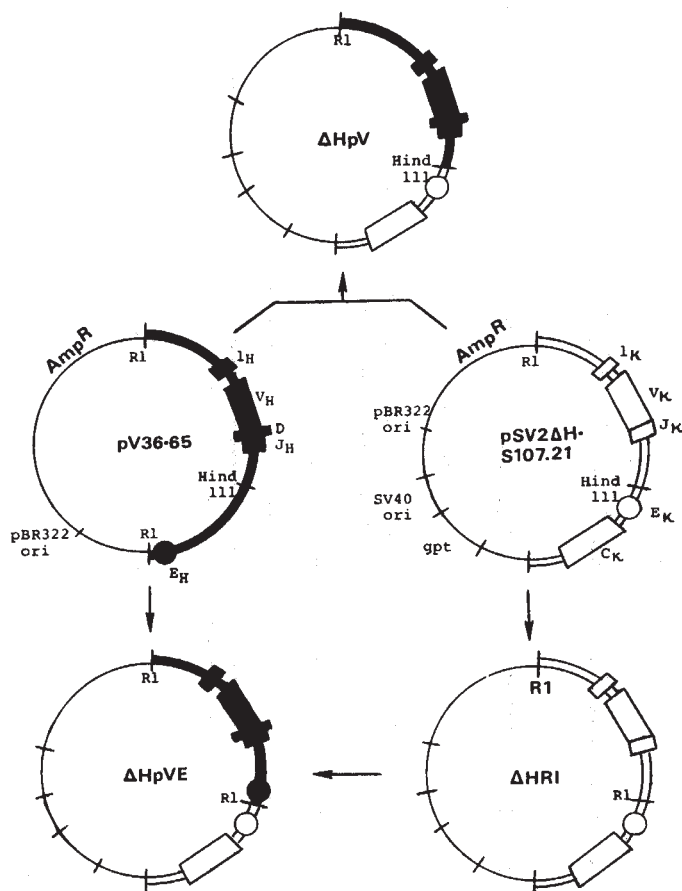


Fig. 1 Construction of $V_H C_\kappa$ -containing plasmids. pV36-65 contains the 363-base pair (bp) rearranged V_H gene derived from the hybridoma cell line 36-65. This gene is flanked by 2.4 kilobase pairs (kbp) of upstream sequence and 2.1 kbp of downstream sequence terminating in the J_H-C_κ intron⁸. pSV2ΔHS107.21 was derived from pSV2-S107 (ref. 16) by elimination of one *Hind*III site. pSV2ΔHS107.21 contains the *Escherichia coli* xanthine-guanine phosphoribosyl transferase gene (*gpt*), and the entire rearranged genomic κ light-chain gene of the S107 myeloma cell line⁹ including the leader (1_κ), V_κ and C_κ exons as well as flanking 5' and 3' sequences. The *gpt* gene confers on mammalian cells resistance to mycophenolic acid when xanthine is present in the medium^{6,7}. The simian virus 40 (SV40) origin of DNA replication (*ori*) and early promoter are located 5' to the *gpt* sequence. The S107 κ gene is oriented in the opposite direction to *gpt*. ΔHR1 was constructed from pSV2ΔHS107.21 by converting the *Hind*III site into an *Eco*RI site. To generate ΔHpV the *Eco*RI-*Hind*III fragment containing the rearranged V_H gene from pV36-65 was substituted for the *Eco*RI-*Hind*III fragment containing the rearranged V_κ gene in pSV2ΔHS107.21. To generate ΔHpVE the *Eco*RI-*Eco*RI fragment containing the rearranged V_H gene from pV36-65 was substituted for the *Eco*RI-*Eco*RI fragment containing the rearranged V_κ gene in ΔHR1. The heavy-chain exons and introns are represented by solid boxes and lines; the κ chain exons and introns are represented by open boxes and lines; the heavy-chain enhancer sequence (E_H)¹²⁻¹⁴, and the κ chain enhancer sequence (E_κ)^{10,11} (V.T.O. and S.L.M., unpublished results) are represented by a solid and an open circle, respectively. Plasmids are not drawn to scale.

the V_H protein sequence^{3,18}. Whereas 6 out of 10 ΔHpVE transformants produced a protein reactive with the anti- κ and anti-Id antisera, only one out of 10 ΔHpV transformants produced such a protein. The relative amounts of the chimaeric protein produced by ΔHpV and ΔHpVE transformants were not significantly different (not shown). The greater number of positive transformants obtained with ΔHpVE might reflect the presence of the heavy-chain enhancer in ΔHpVE.

The ΔHpV positive transformant (which was the first to be recovered) was subcloned in soft agarose^{19,20}, and one clone, L9E84, was selected for further studies. Figure 2a shows an

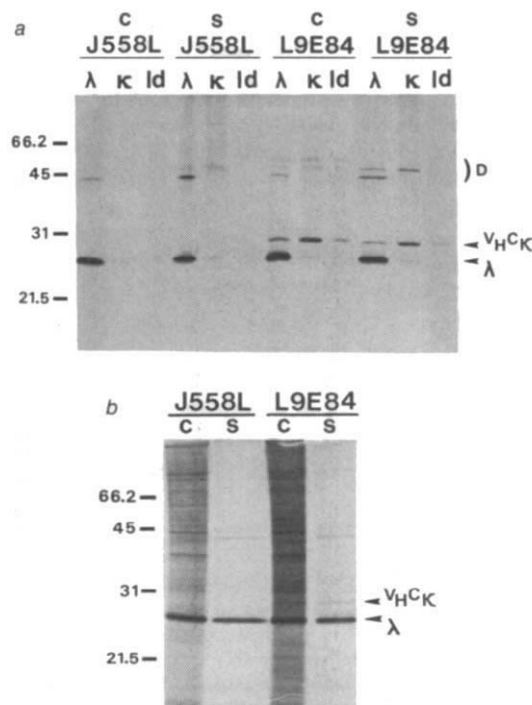


Fig. 2 Autoradiographs of SDS-polyacrylamide gels of cytoplasmic (c) and secreted (s) proteins. Cells were biosynthetically labelled with ³⁵S-methionine in methionine-free Dulbecco's modified Eagle's medium (Irvine Scientific, Santa Ana, California) for 1 h for cytoplasmic protein assay and 3 h for secreted protein assay. Cell extracts were in 0.5% Nonidet P-40 (NP40) containing 0.06 M iodoacetamide. Electrophoresis was in 12.5% SDS-polyacrylamide gels in non-reducing conditions. The positions of the molecular weight markers (Biorad) (in kilodaltons) are indicated; the positions of $V_H C_\kappa$ and λ light chain are indicated by arrows. **a**, Cytoplasmic and secreted proteins from J558L and from L9E84 cells were immunoprecipitated with rabbit anti-mouse antisera and *Staphylococcus aureus*²⁶ (Staph A) and electrophoresed as described²⁷. The antisera used are indicated above each lane. Rabbit anti-mouse κ was obtained from M. Potter under the auspices of NCI contract NO1-CB-25584. Rabbit anti-mouse $\alpha + \lambda$ (designated λ) was prepared in the laboratory of S.L.M. by L. A. Wims. Rabbit anti-Id antiserum was prepared by L. J. Wysocki. Dimers (D) are indicated. **b**, Total cytoplasmic and secreted proteins from J558L and from L9E84 cells. Densitometer tracings of L9E84 lanes were done using a Quick Scan (Helena Laboratories). For quantitation, peaks from Xerox copies of the scans were cut out and weighed.

immunoprecipitation analysis of biosynthetically labelled cells. A protein of the expected molecular weight (29,500) reactive with anti- κ and with anti-Id antisera is present in L9E84 but not in J558L cell extracts. Furthermore, this protein is secreted into the medium (Fig. 2a). Immunoprecipitation of $V_H C_\kappa$ from L9E84 culture supernatant by anti- κ antiserum removed the species reactive with anti-Id antiserum (not shown). The $V_H C_\kappa$ chimaeric protein can be differentiated from the J558L λ light chain by its lower mobility in SDS-polyacrylamide gels in non-reducing conditions (Fig. 2). The upper bands in Fig. 2a are probably covalent dimers (D); from the relative sizes of these putative dimers it seems that $V_H C_\kappa$ can dimerize both with itself and with the J558L λ light chain.

The $V_H C_\kappa$ chimaera comprises about 1% of the biosynthetically labelled L9E84 cell protein and is present in one-tenth the amount of J558L λ light chain, as determined by densitometer tracing of the autoradiogram shown in Fig. 2b.

Ascites from BALB/c mice injected intraperitoneally with L9E84 cells contains the chimaeric protein, as determined by radioimmunoassay. This assay measured the ability of the ascites fluid to compete with the ¹²⁵I-labelled idiotype-bearing molecule, 36-65 Fab, for the binding of rabbit anti-Id antiserum³.

mRNA encoding the $V_H C_\kappa$ protein in L9E84 cells was detected by Northern analysis (Fig. 3). An RNA species of the expected size, ~1.3 kilobases (kb), that hybridizes with an azophenylarsonate (Ars) idiotype probe (specific for the V_H sequence) and with a κ probe (specific for the C_κ sequence) is present in L9E84 but not in J558L cells. It is noteworthy that the chimaeric intron between V_H and C_κ is probably correctly

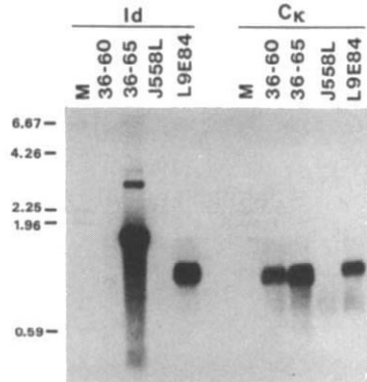
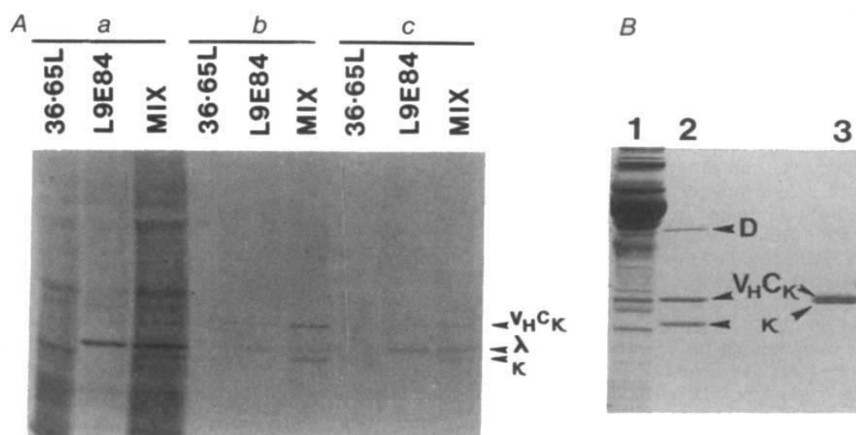


Fig. 3 Northern analysis of L9E84 and control cell RNA. Total cell RNA was prepared from culture cells as described elsewhere²⁸. The RNA (6 µg per lane) was electrophoresed in duplicate in a 1.2% agarose gel in 10 mM Na-phosphate and transferred to nitrocellulose²⁹. The nitrocellulose filter was cut in two and the RNA hybridized to nick-translated ³²P-labelled probes. One half was hybridized to a 133-bp V_H probe specific for the V_H gene encoding the anti-Ars idiotype-positive heavy chain^{8,30}, while the other was hybridized to a mouse C_κ region cDNA probe (obtained from A. Bothwell and D. Baltimore). The cell line from which the RNA was derived is indicated above each lane. 36-60 is an A/J-derived hybridoma which produces an antibody specific for Ars but devoid of the Ars cross-reactive idiotype³, and uses an unrelated V_H gene. A ³²P-labelled *Hind*III digest of phage λ DNA was used as a molecular weight marker (M), and fragment sizes are indicated in kbp. The two halves of the nitrocellulose filter were reattached for autoradiography.

Fig. 4 Association of $V_H C_\kappa$ with 36-65 κ light chain to form an antigen-binding molecule. **A:** *a*, Dialysed cell extracts and mix preabsorbed with BGG-Sepharose. One-fifth the amount used for binding to Ars-BGG-Sepharose was applied to the gel. *b*, Protein bound to Ars-BGG-Sepharose. *c*, Protein bound to Ars-BGG-Sepharose in the presence of 0.5×10^{-3} M Ars-tyrosine (ABA-tyrosine from Bioresearch, San Rafael, California). The positions of $V_H C_\kappa$, λ and 36-65 κ chain are indicated. **B**, SDS-polyacrylamide gel electrophoresis of $V_H C_\kappa$ - $V_\kappa C_\kappa$ dimers purified from 25D3 ascites. Lane 1, 2 µl of ascites, in non-reducing conditions; lane 2, purified $V_H C_\kappa$ - $V_\kappa C_\kappa$ dimers in non-reducing conditions; lane 3, purified $V_H C_\kappa$ - $V_\kappa C_\kappa$ dimers, in reducing conditions. The positions of $V_H C_\kappa$, κ and covalent dimers (D) were determined from their ³⁵S-biosynthetically labelled counterparts in cell line 25D3, and are indicated by arrows.



Methods: **A**, L9E84 and 36-65L cells were biosynthetically labelled separately with ³⁵S-methionine and cell extracts prepared as described in Fig. 2 legend. Portions of 36-65L, L9E84 and an equal mixture of the two extracts (MIX) were made up to 0.5 M in propionic acid to dissociate interchain noncovalent interactions. After 30 min at room temperature the individual extracts and the mix were dialysed exhaustively against 5 mM of Na-acetate pH 5.5 at 4 °C to allow association into dimers^{31,32}, and finally against phosphate-buffered saline (PBS) at room temperature. The dialysed extracts and the mix were preabsorbed with BGG-coupled Sepharose for 2.5 h at 4 °C to reduce nonspecific binding, and then portions of each were mixed with 15 µl of Ars-BGG-Sepharose for 10 h at 4 °C in Eppendorf tubes. The resin aliquots were washed five times with 1 ml of PBS, 1 M NaCl, 0.5% NP40, once with 1 ml of ethylene glycol^{33,34} in the above solution, and once with 1 ml of PBS. Bound protein was eluted with SDS sample buffer at 100 °C³⁵. SDS-polyacrylamide gel electrophoresis in non-reducing conditions, and auto-radiography were as described²⁷. Densitometer tracing and quantitation were done as described in Fig. 2 legend. (The ratio of methionine residues in $V_H C_\kappa$ to those in 36-65 κ is 3:2.) **B**, Ascites was produced in (BALB/c × A/J) F₁ mice by intraperitoneal injection of 10^5 25D3 cells after priming with 0.5 ml of pristane (2,6,10,14-tetramethylpentadecane; Aldrich)³⁶. Dimers with antigen-binding activity were purified by affinity chromatography on Ars-BGG-Sepharose. Elution was with 0.2 M NH₄OH. The eluted material was chromatographed on an Ultrogel AcA 44 column (LKB) that had been calibrated with molecular weight markers; the bulk of the material was recovered as a 55,000-molecular weight protein peak. SDS-polyacrylamide gel electrophoresis was performed as described above, and proteins were visualized by Coomassie blue staining.

spliced using the donor splice site from the heavy-chain gene and the acceptor splice site from the κ light-chain gene. Correct splicing of a chimaeric intron was observed previously²¹.

We wished to determine whether $V_H C_\kappa$ would associate with the homologous 36-65-derived κ light chain to form an antigen-binding dimer; such a dimer would be analogous to a Fab fragment, except that it would have two C_L domains. It has been shown that V_H and V_L can associate to form a fully active antibody-combining site or Fv fragment^{1,2}. Furthermore, X-ray crystallographic evidence suggests that in a light-chain dimer one monomer is oriented as the light chain while the other simulates the heavy chain in an antigen-binding Fab fragment²². It was therefore expected that a $V_H C_\kappa$ - $V_\kappa C_\kappa$ dimer would function as a Fab fragment. For this experiment the source of 36-65 κ chain was cell line 36-65L, a variant of the 36-65 cell line that synthesizes and secretes only the κ light chain and no heavy chain (isolated by J.S.). When $V_H C_\kappa$ and the homologous 36-65 κ light chain were associated *in vitro*, the resulting molecule bound to Ars-bovine γ -globulin (Ars-BGG)-Sepharose. SDS-polyacrylamide gel electrophoresis of the affinity-bound protein (Fig. 4A*b*, MIX) and densitometric analysis showed that the $V_H C_\kappa$ and the 36-65 κ chain were present in a 1:1 molar ratio. Binding to Ars-BGG-Sepharose was significantly reduced by competition with Ars-tyrosine hapten (Fig. 4A*c*, MIX). λ Chain, in amounts of ~30% of the κ or $V_H C_\kappa$ chain, also bound to the Ars-BGG-Sepharose (Fig. 4A*b*, MIX); this binding is evidently nonspecific because, unlike that of the κ and $V_H C_\kappa$ chains, it is not competed for by the Ars-tyrosine hapten (Fig. 4A*c*, MIX). L9E84 cells produce large amounts of this chain (see Figs 2*b*, 4A*a*), and ~2% of the input chain binds to the Ars-BGG resin.

These data suggest that a $V_H C_\kappa$ chimaera can associate with light chain *in vitro* to form an antigen-binding molecule. We estimate, from densitometer tracing of gel autoradiograms (not shown), that 20–30% of the $V_H C_\kappa$ protein had associated with 36-65 light chain to form a functional binding site.

We have recently succeeded in introducing the $V_H C_\kappa$ gene into 36-65L cells by fusion of 36-65L cells with myeloma cells that had been transfected with the $V_H C_\kappa$ gene (J.S., unpub-

lished). This resulted in *in vivo* association of $V_H C_\kappa$ with the light chain of 36-65. Formation of functional antibody molecules *in vivo* following transfection of heavy and light chains into myeloma cells has been demonstrated²³. Ascites from (BALB/c $\times$ A/J)F₁ mice that had been injected with the hybrid cell line, 25D3, contains the $V_H C_\kappa$ - $V_\kappa C_\kappa$ dimer. This dimer was purified by affinity chromatography on Ars-BGG-Sepharose. Gel filtration chromatography indicated a molecular weight of 55,000, as expected. SDS-polyacrylamide gel electrophoresis showed the presence of $V_H C_\kappa$ and covalent dimers (Fig. 4B).

Measurements of the fluorescence quenching of the $V_H C_\kappa$ - $V_\kappa C_\kappa$ dimer and of 36-65 hybridoma protein by Ars-tyrosine were made as described²⁴ and used to calculate K_a . The K_a values for the association of $V_H C_\kappa$ - $V_\kappa C_\kappa$ and that of the 36-65 protein with Ars-tyrosine (assuming one binding site per molecule for the $V_H C_\kappa$ - $V_\kappa C_\kappa$ dimer and two binding sites per molecule for the 36-65 protein) are essentially the same: $3.8 \times 10^5 \text{ M}^{-1}$ and $4.2 \times 10^5 \text{ M}^{-1}$, respectively. A similar value was determined previously for hybridoma protein 36-65 (ref. 24).

It would be interesting to compare X-ray crystallographic studies of the $V_H C_\kappa$ - $V_\kappa C_\kappa$ dimer and of the Fab fragment of 36-65 protein. The dimer might be a good candidate for crystallization because of its increased symmetry compared with Fab fragments. (It has been observed that some Fab fragments cannot be induced to crystallize²⁵.) Such structural studies might provide insights into designing antibody molecules consisting of V_H and V_L attached to other molecules of interest such as enzymes and toxins.

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Schwann cells induce morphological transformation of sensory neurones *in vitro*

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Cell-cell interactions are thought to play a crucial part in determining the developmental fate of vertebrate cells and regulating their subsequent differentiation. In the peripheral nervous system, for example, signals from neuronal axons determine whether or not some Schwann cells wrap their plasma membrane concentrically around the axon to form a myelin sheath^{1,2}. Moreover, there is some evidence that the interactions between Schwann cells and neurones are not all one way: for example, Schwann cells are thought to provide signals for neuronal sprouting and regeneration³⁻⁵. However, there are no clear examples in which Schwann cells have been shown to influence the normal development of neurones. Here I have used purified populations of embryonic sensory neurones and Schwann cells to demonstrate that Schwann cells have a dramatic influence on the development of these neurones. In the presence of Schwann cells, but not other cell types, the sensory neurones undergo a morphological transformation from an immature bipolar form to a mature pseudo-unipolar form. This provides a striking example of the importance of glial cells for neuronal development.

During development in the dorsal root ganglion (DRG) of birds and mammals, sensory neurones are initially bipolar in form, extending one axon to the periphery and the other to the spinal cord. During the second half of development, these neurones undergo a remarkable change in morphology well described by Ramon y Cajal⁶. The cytoplasm of the neuronal cell soma bulges to one side and the two axons approach one another so that the cell becomes bell-shaped. Eventually the cytoplasm between the cell body and the processes elongates to form the initial segment of the axon from which the original processes emanate (see Fig. 1). This change from bipolar to pseudo-unipolar morphology is associated with a rearrangement of the cell body cytoplasm and cytoskeleton^{7,8}.

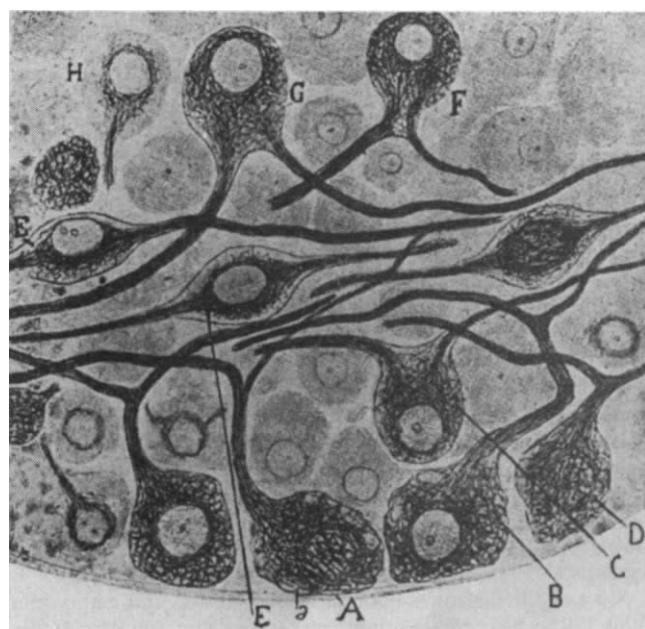
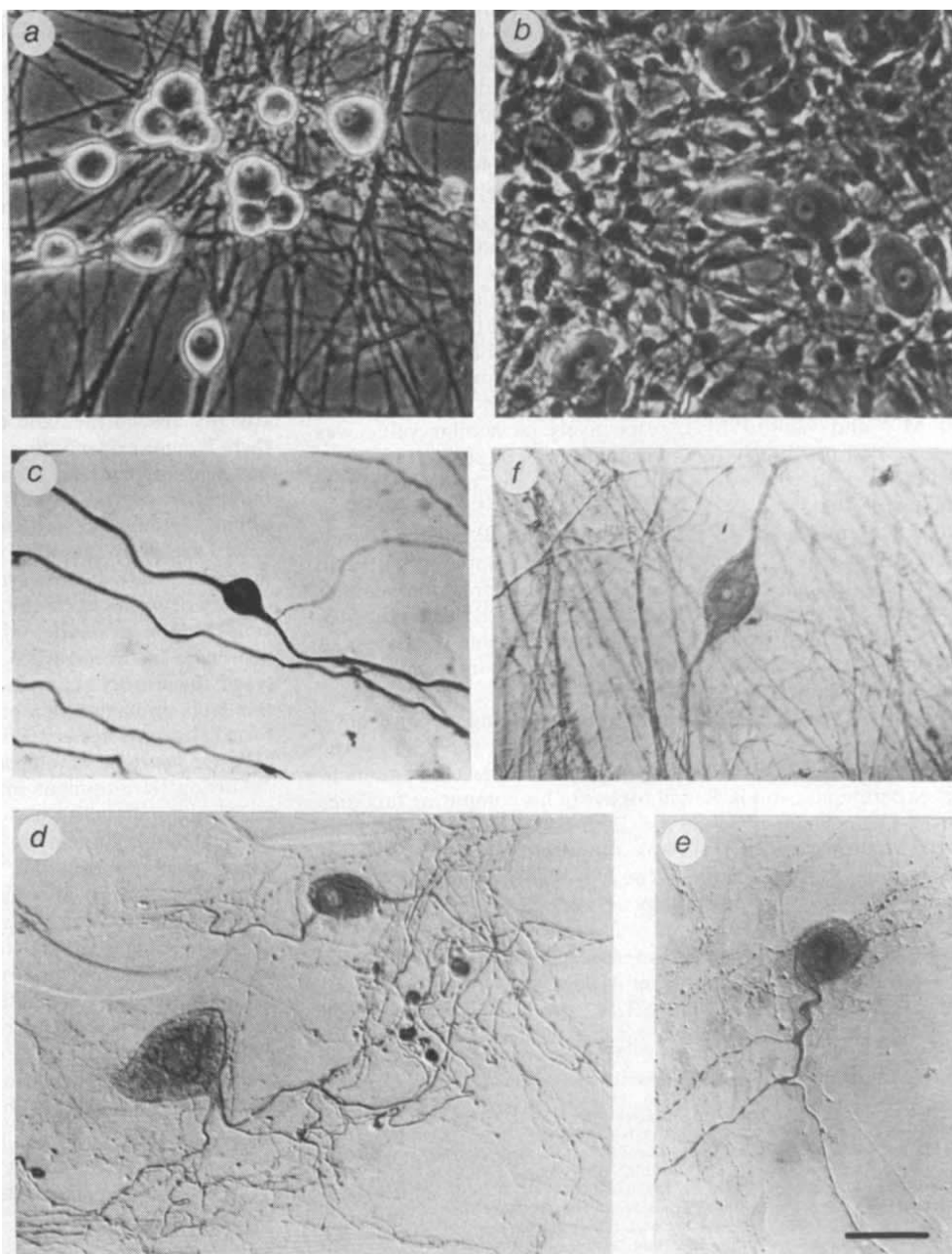


Fig. 1 DRG neurones from 14-day-old chick embryo, stained with silver nitrate. This drawing which shows the transition of bipolar sensory neurones to the pseudo-unipolar form is reproduced from ref. 6.

Fig. 2 *a, b*, Phase-contrast micrographs of sensory neurones grown for 10 days without the addition of Schwann cells (*a*), or grown for 5 days after the addition of Schwann cells (*b*). *c*, Sensory neurone grown for 15 days on a collagen substrate in the absence of non-neuronal cells. *d*, Sensory neurones 10 days after the addition of Schwann cells. The neurones appear to be undergoing a transition to the pseudo-unipolar form. *e*, Sensory neurone 10 days after the addition of Schwann cells. *f*, Sensory neurone grown on extracellular matrix material laid down by bovine endothelial cells. Scale, 50 μ m.

Methods: *a, b*, DRGs from 9–10-day-old chick embryos were dissociated using 0.1% Dispase (Boehringer Mannheim) in Ca^{2+} , Mg^{2+} -free saline and plated onto collagen-coated 35-mm tissue culture dishes (Falcon) or 16-mm glass coverslips in 24-multi-well trays (Linbro). Cells were grown in minimal essential medium containing 10% heat-inactivated horse serum, 5% chick embryo extract, 2 mM glutamine, 50 $\mu\text{g ml}^{-1}$ penicillin, 50 U ml^{-1} streptomycin and 1 $\mu\text{g ml}^{-1}$ nerve growth factor as previously described^{9,10}. The cultures were treated with 5×10^{-6} M cytosine arabinoside for 5 days to eliminate non-neuronal cells. At this time, purified Schwann cells were added to some of the neurone-alone cultures. The plating density of the Schwann cells was either 200,000 per 35 mm dish or 50,000 per 19 mm multi-well with coverslip. *c–f*, Sensory neurones plated at low density and labelled with anti-NF antibody viewed with Nomarski optics. Cultures were fixed with 4% paraformaldehyde followed by extraction with cold methanol. The extracted cells were then incubated with a monoclonal antibody (RT 97)¹² to neurofilament proteins, washed and then incubated with anti-mouse Ig conjugated to peroxidase (Tago). The bound antibody was then visualized by reacting with diaminobenzidine (0.05%) and H_2O_2 (0.01%).



In the present experiments, DRGs were dissected from 9–10-day chick embryos, dissociated to single cells and plated onto collagen-coated glass coverslips or plastic tissue-culture dishes. The cultures were treated for 5 days with cytosine arabinoside (5×10^{-6} M) to kill most of the ganglionic non-neuronal cells^{9,10}. After this period purified Schwann cells were added to some of the neuronal cultures. The Schwann cells were obtained from newborn rat sciatic nerves and purified free of fibroblasts by pulsing with cytosine arabinoside (10^{-5} M for 2 days) followed by complement-mediated cell lysis of Thy 1.1-positive fibroblasts according to the method of Brookes *et al.*¹¹. After growth for a further 5–15 days, the cultures were photographed, then fixed and labelled with a monoclonal antibody against neurofilament proteins (RT97; anti-NF)¹².

Most of the neurones in cultures of 9–10-day-old embryonic chick DRGs were phase bright and bipolar, and maintained this morphology for periods of up to 1 month if grown in the absence of non-neuronal cells (Fig. 2*a, c*). However, when purified Schwann cells were added, the morphology of the neurones changed dramatically within 5 days (see below). When viewed

with phase-contrast optics, all the neurones had become flat and phase-dark; moreover, the initial segment of the axons appeared thicker (Fig. 2*b*). Labelling the mixed cultures with anti-NF antibody showed that all the neurones had changed from the bipolar to the pseudo-unipolar form. Figure 2*c–e* shows neurones grown at low density with and without the addition of Schwann cells and labelled with anti-NF. This set of cultures was chosen for illustration because they clearly show the intermediate forms reminiscent of Ramon y Cajal's drawings (Fig. 2*d*).

The time required for this change to occur was related to the number of Schwann cells added: with high densities of Schwann cells the change occurred in less than 1 week, the first 'flattening' being observed after 3 days. With low numbers of Schwann cells, the change occurred after a longer period (up to 15 days); however, when the neurones eventually flattened, the majority did so over a period of a few days. Although the dependence on Schwann cell number and the synchrony of the transition suggested that a secreted factor might be responsible for the effect, attempts to induce the morphological change with super-

natants from Schwann cell cultures were unsuccessful. This suggests that neuronal-Schwann cell contact may be required.

Of the cells tested, only Schwann cells were able to induce the morphological change in DRG neurones. Fibroblasts from either rat or chick skin, chick myotubes, rat cortical astrocytes, or the glial cell lines RN22 and C6, had no effect on neuronal morphology when added to DRG cultures. Changing the composition of the substrate by adding fibronectin and/or laminin to the collagen also had no effect. Similarly, DRG neurones cultured on the extracellular matrix material laid down by bovine endothelial cells¹³ remained in the bipolar form after 2 weeks, although this matrix greatly stimulated neurite extension. (Fig. 2f). Therefore if extracellular matrix components are involved in the change, these components seem to be specific to the Schwann cells.

The present experiments used early embryonic chick neurones and purified rat sciatic nerve Schwann cells. Fortunately, the mechanism of this signalling between Schwann cells and neurones seems to be conserved between species. Chick rather than rat DRG neurones were studied because of the relative ease of culturing early embryonic chick neurones free of non-neuronal cells; many of the neurones taken from chick embryos older than 10 days (or rat embryos older than 15 days) have already been signalled to become pseudo-unipolar, and when put into culture retain this morphology. Rat Schwann cells were used because of the availability of methods for identifying and purifying these cells; comparable methods for identifying and purifying chick Schwann cells are not available. However, I have found that chick non-neuronal cells enriched for glial cells (using spindle-shape morphology as a criterion) also promote the morphological transformation of chick sensory neurones. Dichter and Fischbach¹⁴ noted the presence of a few 'flat' phase-dark chick DRG neurones, which were probably pseudo-unipolar, in cultures grown in the presence of ganglionic non-neuronal cells that were mostly mesenchymal rather than glial cells. Other workers¹⁵ have described the presence of pseudo-unipolar sensory neurones in unpurified rat DRG cultures containing both rat neurones and Schwann cells. However, because of the heterogeneity of the cell populations used, no specific interaction between two identified cell types could be identified in any of these three cases.

The present report illustrates the power of using purified cells to determine which of the properties of differentiated neurones are intrinsic to these cells and which depend on interaction with other cell types. There is a striking similarity between the transformation seen in these cell mixing experiments *in vitro* and that seen during the normal development of sensory neurones *in vivo*. This, together with the fact that the transformation occurs at a time that correlates with Schwann cell proliferation in the DRG¹⁶, suggests that Schwann cells play a crucial part in mediating this morphological change *in vivo* as well as *in vitro*.

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Epstein-Barr virus transforms precursor B cells even before immunoglobulin gene rearrangements

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The very early stages of the human B-cell differentiation pathway are poorly understood, primarily because of the lack of appropriate permanent cell lines. Epstein-Barr virus (EBV) is a putative human oncogenic virus which transforms human B cells *in vitro* into continuously proliferating cells¹. It has been believed that EBV transforms mature B cells^{2,3} but recently, transformation of immature pre-B-cell lines has been reported^{4–6}, suggesting that EBV might also transform cells much earlier in the B-cell lineage. We report here the establishment of cell lines transformed by EBV at various stages of the B-cell differentiation pathway. Interestingly, two lines showed the complete absence of immunoglobulin synthesis and the lack of immunoglobulin gene rearrangement despite containing EBV genome and surface markers of B cells. Our results indicate that EBV can infect and transform cells of the B lymphocyte lineage even before immunoglobulin gene rearrangement.

EBV was obtained from the culture fluid of the B95-8 marmoset cell line⁷. The fetal liver was obtained from an embryo artificially aborted at the 16th week of gestation. Mononuclear cells separated by Ficoll-Conray gradient centrifugation were infected with EBV at a multiplicity of infectivity of 0.1. Infected cells were distributed into microculture plates (no. 3042, Falcon) at a density of 1×10^5 cells per well in RPMI 1640 medium supplemented with 20% heat-inactivated fetal bovine serum, 0.2 mM L-serine, 1 mM pyruvic acid, 2 mM L-glutamine, 100 U ml⁻¹ of penicillin, 100 µg ml⁻¹ of streptomycin and 10 mM HEPES and were cultured at 37 °C in an atmosphere of 5% CO₂ in air⁸. After 30–40 days, transformation occurred in 30% of the wells, as determined by the morphological appearance of lymphoblastoid cells, increase of cell numbers and acid production.

The immunoglobulin phenotypes of 24 cell lines thus obtained were examined (Table 1). The various cell lines showed different immunoglobulin production and were classified into four groups. Of the 24 cell lines, 20 were IgM producers which had both cytoplasmic and surface μ and light chains. The amounts of IgM secreted in culture fluid varied over 270–52,500 ng ml⁻¹. The light chains made by all of these cell lines except FLEB5 were κ isotype. This finding may reflect the dominant expression of κ isotype in the fetal liver B cells, and supports the hypothesis that the κ -chain gene is activated before the λ -chain gene in human B-cell ontogeny⁹. Another cell line, FLEB12, synthesized only μ chains in the absence of light chains and therefore appeared to represent a pre-B cell^{10,11}. Using the method of double colour staining¹², 60% of the FLEB12 cells possessed μ chains only in the cytoplasm but 10% had μ chains both in the cytoplasm and on the cell surface (data not shown). This result supports the proposal that μ and light chains may not need to be assembled for expression on the cell surface. In the case of line FLEB20, only κ chains were detected. This might be a similar cell line to those mouse cell lines reported by Siden *et al.*¹³ which produce light chains only but have rearranged heavy-chain genes¹⁴. Unfortunately, the FLEB20 was subsequently lost. The remaining two cell lines, FLEB14 and 18, showed complete absence of immunoglobulin synthesis.

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Table 1 Immunoglobulin phenotypes of cell lines

Cell lines	Secreted immunoglobulin (ng ml ⁻¹)		Surface immunoglobulin (%)						Cytoplasmic immunoglobulin (%)					
	IgM	IgG	μ	γ	δ	α	λ	κ	μ	γ	δ	α	λ	κ
FLEB 1	820	0	0.6	0	0	0	0	0.2	0.8	0	0	0	0	0.7
2	15,000	0	37	0	0	0	0	39	50	0	0	0	0	57
3	550	0	0.8	0	0	0	0	0.4	1.2	0	0	0	0	0.5
4	1,950	0	2	0	0	0	0	1	3	0	0	0	0	0.5
5	12,000	0	14	0	0	0	16	0	25	0	0	0	19	0
6	270	0	0.8	0	0	0	0	0.1	0.4	0	0	0	0	0.1
7	350	0	1	0	0	0	0	0.3	0.9	0	0	0	0	0.8
8	2,460	0	5	0	0	0	0	2	6	0	0	0	0	3
9	890	0	0.1	0	0	0	0	0.6	1.5	0	0	0	0	0.3
10	1,400	0	0.2	0	0	0	0	0.1	0.2	0	0	0	0	0.1
11	750	0	1	0	0	0	0	2	1	0	0	0	0	2
13	1,300	0	1	0	0	0	0	2	1	0	0	0	0	0.8
15	290	0	0.1	0	0	0	0	0.1	0.3	0	0	0	0	0.2
16	48,000	0	52	0	0	0	0	40	38	0	0	0	0	41
17	280	0	0.7	0	0	0	0	0.8	1	0	0	0	0	0.6
19	2,500	0	2	0	0	0	0	2	2	0	0	0	0	0.8
21	440	0	0.4	0	0	0	0	1	0.7	0	0	0	0	2
22	52,500	0	38	0	0	0	0	22	33	0	0	0	0	27
23	680	0	1	0	0	0	0	2	3	0	0	0	0	1
24	540	0	0.6	0	0	0	0	0.2	0.8	0	0	0	0	0.1
12	57	0	11	0	0	0	0	0	66	0	0	0	0	0
20	0	0	0	0	0	0	0	8	0	0	0	0	0	11
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0	0

The amounts of IgG and IgM were determined by an inhibition radioimmunoassay described by Platts-Mills and Ishizaka²⁴. Briefly, immunoadsorbent column-purified IgG and IgM were labelled with ¹²⁵I(NEN) by the chloramine-T method. 100 μ l of appropriately diluted supernatants and standard human IgG (or IgM) were mixed with 0.1 ml of rabbit anti-human IgG (or anti-human IgM) serum. After 2 h incubation at room temperature, 0.1 ml of ¹²⁵I-labelled IgG (or IgM) (59 ng ml⁻¹) was added and incubated for another 2 h at room temperature. Then 0.1 ml of goat anti-rabbit IgG serum was added and allowed to stand overnight at 4 °C. The precipitates were spun down and radioactivity was counted using an autogamma-counter. Cytoplasmic and surface immunoglobulins were detected by direct immunofluorescence. The cells were smeared on a coverslip, fixed in acetone at -20 °C and stained for cytoplasmic immunoglobulin with fluorescein isothiocyanate (FITC)-coupled rabbit monospecific antisera, F(ab')₂ fragment, against human μ , δ , γ , α , κ and λ chains. Surface immunoglobulin was stained by the method of Kumagai *et al.* after treatment with acid pH²⁵.

Three representative cell lines (FLEB2, 12 and 14) were subcloned in semi-solid agarose. Each subclone conserved the same characteristics as the parental cell lines. To confirm the immunoglobulin phenotypes of three subcloned lines (FLEB2, 12 and 14), the cells were metabolically labelled with ¹⁴C-leucine and their cell lysates and culture fluids were immunoprecipitated with purified anti- μ serum. The precipitated polypeptides were analysed by SDS-polyacrylamide gel electrophoresis (PAGE) (Fig. 1). In the lysate (lane 1) and culture fluid (lane 4) of FLEB2 the molecular weights (MWs) of two observed polypeptides were 76,000 and 29,000, corresponding to those of authentic μ and light chains purified from human serum. In the case of FLEB12 (Fig. 1, lanes 2, 5), two μ -related polypeptides were detected in the lysate but no precipitable polypeptides were observed in the culture fluid. The minor band of the lysate was 76,000 MW and identical in size to the standard μ chain, while the major one was ~80,000 MW. This higher molecular weight may result from differences in the length of these polypeptides and/or in the degree of glycosylation. Alternatively, it may not be a μ -gene product, such as the supposed heavy-chain binding protein (BiP) noncovalently bound to heavy chains reported in murine pre-B-cell lines¹⁵. The anti- μ antibody did not precipitate any molecules at all in the case of FLEB14 (lanes 3 and 6). Thus, FLEB14 was completely lacking in immunoglobulin expression.

To determine whether FLEB14 belonged to the B-cell lineage or not, the lines were tested for various other markers (Table 2). Like the other cell lines tested, FLEB14 possessed Fc μ receptors, C3 receptors and HLA-DR antigens, which are characteristic of B cells, although not unique to them. They lacked any T-cell markers and peroxidase activities. In addition, FLEB14 was positive for EBV-induced nuclear antigens

Table 2 Other various markers of cell lines

	FLEB2	FLEB12	FLEB14
EBNA	+	+	+
Fc μ receptor	8%	24%	27%
Fc γ receptor	0%	0%	0%
C3b receptor	34%	82%	15%
C3d receptor	7%	18%	9%
HLA-DR antigen	+	+	+
SRBC receptor	-	-	-
T-cell antigen			
Leu 1	-	-	-
Leu 2a	-	-	-
Leu 3a	-	-	-
Leu 4	-	-	-
TdT	-	-	-
Peroxidase	-	-	-
CALLA	3%	19%	0%

EBNA was detected according to the method of Reedman and Klein¹⁶. Rosette formation of the cells was performed by the method of Tachibana *et al.*²⁶. Neuraminidase-treated sheep erythrocytes (SRBC) were used for E-rosette formation. Cells with IgG-Fc or IgM-Fc receptors were tested with IgG- or IgM-coated ox erythrocytes. IgM-human complement-coated SRBC and IgM-C5-deficient mouse complement-coated SRBC were used for detection of C3b and C3d receptors. HLA-DR antigen, CALLA(J5)²⁷ and T-cell differentiation antigens (Leu 1, Leu 2a, Leu 3a, Leu 4) were tested by fluorocytometry using murine monoclonal antibodies (Becton Dickinson). Terminal deoxynucleotidyl transferase (TdT) activity was determined according to the method of Tofen *et al.*²⁸. Peroxidase staining was performed by a standard method.

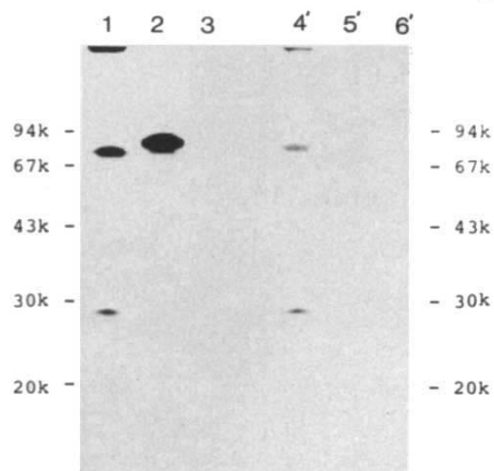


Fig. 1 Lanes 1, 2 and 3 are from cell lysates of FLEB2, 12 and 14. Lanes 4, 5 and 6 are from culture supernatants of FLEB2, 12 and 14. Cells were cultured at 5×10^6 cells per ml with $5 \mu\text{Ci ml}^{-1}$ of ^{14}C -leucine (NEN) for 2–12 h in leucine-free minimal essential medium supplemented with 10% heat-inactivated fetal calf serum. After labelling, the culture media were saved. The cells were washed twice with cold phosphate-buffered saline and were lysed in lysis buffer containing 10 mM sodium phosphate pH 7.4, 0.1 M NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 1 mg ml^{-1} of bovine serum albumin and 1 mM phenylmethylsulphonyl fluoride. The lysed cells were centrifuged at 100,000g for 60 min at 4°C and the supernatants were saved. The spent media and cell lysates were mixed with 10 μl of rabbit anti-human IgM serum. After 12 h incubation at 4°C , 0.1 ml of a 10% suspension of fixed *Staphylococcus aureus* Cowan I was added and incubated for 2 h at room temperature followed by three washings. Bound immunoglobulins were removed and reduced in buffer containing 10 mM Tris-HCl pH 6.8, 6.2 M urea, 2.3% SDS and 5% 2-mercaptoethanol for 3 h at 70°C , and *S. aureus* was removed by centrifugation. Reduced samples were separated on a 10–15% gradient polyacrylamide gel according to the method of Laemmli²⁹. Following electrophoresis, the gel was fixed and fluorographed.

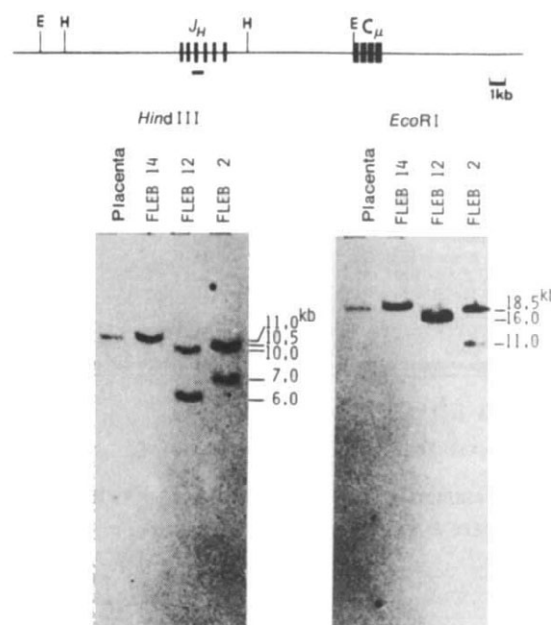


Fig. 2 Immunoglobulin heavy-chain gene analysis of FLEB2, 12 and 14 cell lines with a cloned human J_H gene fragment as a probe. High molecular weight DNA was isolated from each cell line according to the method of S. Gattoni (personal communication)³⁰. The human placental DNA was used as a control. The fragments obtained by *Hind*III or *Eco*RI digestion of DNA (about 2 μg) were size-fractionated by electrophoresis in a 0.5% agarose gel, transferred to a nitrocellulose filter according to the method of Southern³¹, then hybridized with a ^{32}P -labelled cloned J_H gene probe (specific activity 3×10^8 d.p.m. per μg). The probe used was a 0.7-kb *Hha*I–*Hha*I fragment of Ch4A.H.Ig μ -24 (ref. 22) which is indicated by a bold bar below the restriction map. The labelling of the probe was performed by nick-translation as previously described³². In the restriction map, structural genes are indicated by closed rectangles, and restriction sites of *Hind*III and *Eco*RI are shown by H and E, respectively.

(EBNA)¹⁶, thus they must have receptors for EBV¹⁷. It has been suggested that the presence of EBV receptor could be considered as a B-cell marker^{17,18}. The common acute lymphoblastic leukaemia antigen (CALLA), which is limited to expression mainly on pre-B cells¹⁹, was not detected on FLEB14 cells. Therefore, FLEB14 appears to be committed to the B-cell lineage but definitive assignment can be made only if immunoglobulin production can be induced.

As the first event characteristic of B-cell ontogeny is the rearrangement of immunoglobulin genes, it was of interest to know the immunoglobulin gene organization of FLEB14. It has been demonstrated that heavy-chain gene rearrangement precedes that of light-chain genes, and is accomplished by the joining of V_H , D and J_H gene segments^{20,21}. The DNA fragments containing J_H before rearrangement, digested by *Hind*III or *Eco*RI, are 11.0 or 18.5 kilobases (kb)²² in length, respectively. The DNAs from cells of three of these lines (FLEB2, 12 and 14) and human placental cells as germ-line control were digested with *Hind*III or *Eco*RI (Fig. 2). *Hind*III digests of FLEB2 gave J_H positive fragments of 10.5 and 7.0 kb and from FLEB12, fragments of 10.0 and 6.0 kb. None of these corresponded to the fragment of the human placental DNA (11.0 kb). FLEB14, however, showed the fragment of the germ-line size. Similarly in *Eco*RI digestion, J_H -positive fragments of 18.5 and 11.0 kb in FLEB2 and 16.0 kb in FLEB12 were detected, indicating that the immunoglobulin genes had undergone rearrangement in these cell lines. *Eco*RI-digested DNA of FLEB14, however, again produced only one band of 18.5 kb, identical in size to that found in the human placental DNA. These results show that somatic rearrangement of the heavy-chain gene has already occurred in FLEB2 and 12, but that in FLEB14 the heavy-chain gene retains its germ-line configuration. Based on other experi-

ments, the light-chain genes also conserve the embryonic configuration in FLEB14 (M.O., unpublished results).

These cell lines should therefore provide a useful model for studying the regulation of B-cell differentiation. Of particular interest is one cell line, FLEB14, which makes neither heavy nor light chain and has no immunoglobulin gene rearrangement despite carrying B-cell markers. This line may be the first example of the category of 'ur-B cell', proposed recently by Wabl *et al.*²³. If immunoglobulin gene rearrangement in FLEB14 can be successfully induced, it will provide a system in which to study the earliest molecular aspects of immunoglobulin gene activation. In preliminary experiments, we have obtained some evidence suggesting heavy-chain gene reorganization in FLEB14 during successive cultivation (M.O., to be published).

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Relaxin affects the central control of oxytocin release

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In several species the myometrium is quiescent shortly before parturition¹. At this time high titres of relaxin are present in the plasma² and there is evidence³ that the hormone has a direct inhibitory action on the uterine muscle. Relaxin could also contribute to uterine quiescence by inhibiting oxytocin release. To determine whether relaxin has a central action on the release of oxytocin, we have studied the effect of intravenous injections of porcine relaxin on milk ejection in the anaesthetized lactating rat. We report that reflex milk ejection was suppressed by relaxin in a dose-dependent manner, the onset of inhibition being rapid and lasting from 10 to 60 min. After the period of inhibition the normal temporal pattern of reflex milk ejection was resumed. Mammary sensitivity to exogenous or endogenous oxytocin was reduced by relaxin but not sufficiently to explain the effects observed. Furthermore, relaxin (1 µg per rat) injected into the cerebral ventricles profoundly disturbed the pattern of reflex milk ejection without affecting the response of the mammary gland to oxytocin. These results suggest a novel role for relaxin within the central nervous system. The site in the brain at which the effects of relaxin are exerted remains unknown.

Rats were taken from the departmental colony where they had been housed in an environment of controlled light and temperature, with water and food available *ad libitum*. The day of delivery was denoted as day 1, and the animals were used between days 8 and 12 of lactation. The protocol for these experiments has been described in detail elsewhere⁴. Briefly, the young rats were separated overnight from their mother to promote mammary engorgement. The following morning the lactating rats were anaesthetized with ethyl carbamate (1.25 g per kg intraperitoneal, i.p.; urethane, BDH) and 2.5 mg intramuscular (i.m.) xylazine (Rompun; Bayer). A saphenous vein and two caudal mammary glands were cannulated and the animal was left for 3 h to stabilize after surgery. Ten hungry young rats were then allowed to suck at the nipples—this stimulus caused the onset of reflex milk ejection characterized by an abrupt and transient rise in intramammary pressure⁵, together with the behavioural response of the sucking young. The latency to the first milk ejection was 12 min (mode) with a range of 5–48 min and thereafter occurred every 3–7 min. After at least eight milk ejections had been observed as a control, purified porcine relaxin, prepared by the method of Walsh and Niall⁶, was given in 0.2 ml isotonic saline by intravenous (i.v.) injection. Three doses of relaxin were tested (2.5, 5 and 10 µg). Milk ejection was considered to be inhibited if intervals between successive milk ejections were significantly longer than intervals in the pretreatment period (see Fig. 1 for statistical analysis).

Injection of relaxin at all three doses cause a clear suppression of reflex milk ejection (Fig. 2); this effect was dose-dependent

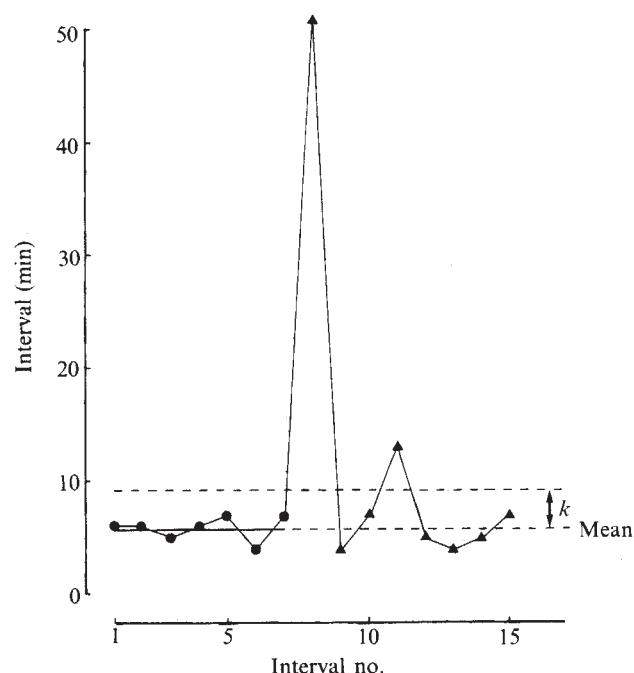


Fig. 1 Analysis used to assess the inhibition of reflex milk ejection by relaxin. This approach was first described by Clarke *et al.*⁴. Intervals between successive milk ejections are plotted sequentially, before (●) and after (▲) injection of relaxin. The lower horizontal line is the mean interval between milk ejections before treatment. The upper dashed line represents a 99% probability estimate ($P = 0.01$) based on the equation $k = \text{s.d.} \times t \sqrt{[(n+1)/n]^*}$ where s.d. = standard deviation of the milk ejection interval before treatment, n = number of control intervals; $t = t'$ -value for $n-1$ degrees of freedom at $P = 0.01$ and $*$ = Bessel's correction for small size of preinjection sample. Milk ejection was judged to have been significantly inhibited if any milk ejection interval, within 30 min of relaxin treatment, exceeded the calculated level above.

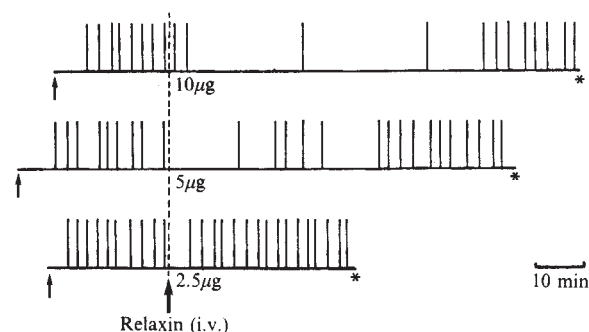


Fig. 2 The effects of intravenous injection of porcine relaxin on the pattern of reflex milk ejection in three suckled, urethane-anaesthetized rats. Each vertical bar corresponds to an intramammary pressure cycle characteristic of milk ejection (↑, onset of sucking stimulus of 10 young rats; *, termination of experiment). The three traces have been aligned with respect to the time of administration of relaxin. Reflex milk ejection was suppressed in a dose-dependent manner (see Table 1).

(Table 1) and lasted from 10 to over 60 min from the time of injection. During periods of inhibition the sensitivity of the mammary gland to oxytocin was tested by i.v. injections of 0.5 mU oxytocin. On all occasions the gland responded to the oxytocin but sometimes after application of 5 or 10 µg relaxin, the amplitude of the pressure response was reduced by up to 50% (Fig. 3), although it was never abolished. After the period of inhibition, reflex milk ejections recommenced with a frequency similar to pretreatment rates. A second dose of relaxin (5 µg) was administered at least 2 h after the initial treatment in four animals; inhibition of the reflex still occurred but to a lesser degree. This might reflect a tachyphylactic response similar to that reported for the myometrium *in vitro*⁷.

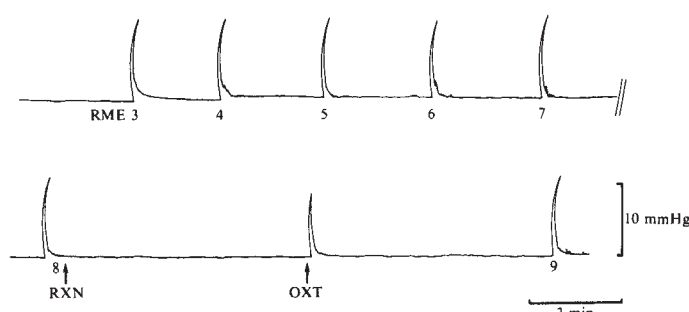


Fig. 3 The effect of relaxin on reflex milk ejection (RME) in an anaesthetized rat. The two traces of intramammary pressure responses are continuous. After the eighth RME, 10 μ g relaxin ($\uparrow$ RXN) was injected i.v. Reflex milk ejection was suppressed for 16 min but during this time the mammary gland was still responsive to 0.5 mU exogenous oxytocin ($\uparrow$ OXT), suggesting that treatment with relaxin affected the central release of oxytocin.

We tested the effect on reflex milk ejection of relaxin injected directly into the ventricular system of the brain in six animals. Rats which had been anaesthetized and prepared as described above were placed in a stereotaxic frame and a microsyringe (Hamilton 701N) positioned with its tip in one of the lateral cerebral ventricles. The tip was confirmed to be in the ventricular system by dye injection at the end of the experiment. Reflex milk ejection was initiated by the sucking young and after eight milk ejections, 1 μ g relaxin in 1 μ l isotonic saline was injected into the ventricle. Relaxin caused a profound disruption in the pattern of milk ejection (Fig. 4) but did not affect the response of the mammary gland to exogenous oxytocin in all six rats. Control injections of 1 μ l isotonic saline ($n=6$) did not affect the temporal pattern of reflex milk ejection. These data imply that the major component of the inhibitory effect of relaxin on milk ejection is exerted within the brain.

Control injections of 0.2 ml isotonic saline (two rats) and an extract of pig ovary (5 μ g) shown to possess no relaxin immunoreactivity or bioactivity (uterine strip assay) did not alter the interval between reflex milk ejections in two and three rats respectively. Evidence suggests that electroencephalogram (EEG) synchrony may be a prerequisite for reflex milk ejection in the rat^{8,9}. Therefore, we recorded the EEG activity in two anaesthetized lactating rats before and after the i.v. administration of relaxin (5 μ g); in neither animal did relaxin alter the EEG activity, suggesting that the hormone did not change the animal's level of arousal. It is also unlikely that the effect of relaxin could be attributed to an action on blood pressure

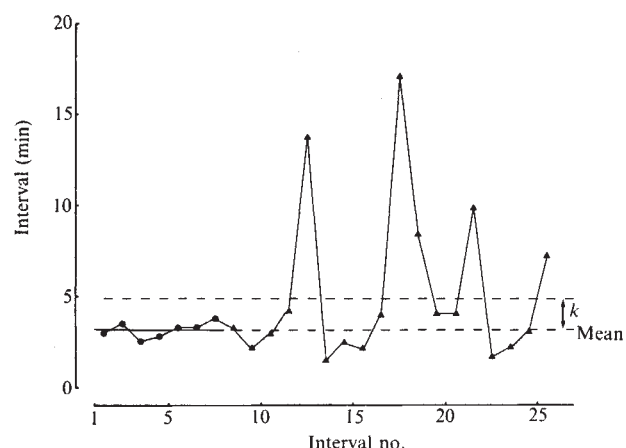


Fig. 4 The effect of intracerebroventricular relaxin on reflex milk ejection. Intervals between successive milk ejections are plotted sequentially before (●) and after (▲) injection of 1 μ g relaxin. The lower dashed line is the mean interval between milk ejections before treatment and the upper dashed line represents 99% probability estimation of significant inhibition of the reflex. Reflex milk ejection was clearly suppressed after central administration of relaxin.

because previous experiments¹⁰ demonstrated that relaxin in doses of up to 20 μ g had no effect on arterial pressure.

A possible explanation of the results is that sensitivity of the mammary glands to oxytocin was diminished by relaxin. Therefore, we examined the effect of exogenous oxytocin on intramammary pressure in lactating rats before and after treatment with relaxin. Each injection of hormone (0.5–1 mU) produced an intramammary pressure cycle with a sharp rise and slow decline. These responses mimic the natural pressure changes seen at reflex milk ejection⁵, and are relatively constant for a particular dose of hormone⁵. At lower doses the responses are variable; for this reason we used a standard dose of 0.5 mU of synthetic oxytocin (Syntocinon; Sandoz). Rats were anaesthetized and cannulated as described above. After 3 h, 0.5 mU oxytocin was injected i.v. and thereafter at 3-min intervals. A stable baseline of at least seven consistent responses was obtained as control in each animal before relaxin was given; the results are shown in Table 2. Only at doses of relaxin of 5 μ g and 10 μ g was partial inhibition recorded (Table 2) yet 2.5 μ g relaxin caused a suppression of reflex milk ejection in 7 out of 10 rats (Table 1), and in all of six rats 1 μ g of the hormone was effective when administered intracerebrally. Furthermore,

Table 1 Effects of porcine relaxin (i.v.) on reflex milk ejection in anaesthetized lactating rats

	Latency (min) to 1st RME [Mode (range)]	RME interval (min) (mean $\pm$ s.e.m.)		No. of rats inhibited	Duration (min) of inhibition [Mode (range)]	Milk ejection ratio (mean $\pm$ s.e.m.)
		Before relaxin	After relaxin			
2.5 μ g Relaxin ($n=10$)	12 (5–34)	4.5 $\pm$ 0.4	5.2 $\pm$ 0.3	7/10	10 (4–16)	3.5 $\pm$ 0.7
5 μ g Relaxin ($n=10$)	10 (7–48)	4.7 $\pm$ 0.8	6.8 $\pm$ 0.7	10/10	14 (6–28)	5.9 $\pm$ 1.6
10 μ g Relaxin ($n=8$)	12 (6–31)	4.7 $\pm$ 0.6	9.9 $\pm$ 0.7	8/8	27 (23–>60)	11.0 $\pm$ 2.3

The statistical analysis used to assess inhibition is illustrated in Fig. 1. RME, reflex milk ejection. The milk ejection ratio is the duration of the longest interval which occurred within 30 min of relaxin treatment divided by the mean interval before treatment.

Table 2 Effects of porcine relaxin (i.v.) on oxytocin-evoked milk ejection

	No. of rats affected	% Reduction in amplitude of IMP [Mean (range)]	Latency (min) to onset [Mode (range)]	Duration of effect (min) [Mode (range)]
2.5 μ g Relaxin ($n=10$)	0/10	—	—	—
5 μ g Relaxin ($n=10$)	2/10	6.2 (4–90)	3 (3–6)	6 (6–12)
10 μ g Relaxin ($n=12$)	6/12	18.6 (4–92)	3 (3–12)	9 (6–18)

A steady baseline of at least seven consistent responses to 0.5 mU oxytocin given at 3-min intervals was obtained as a control before i.v. injection of relaxin. The reduction in amplitude of the intramammary pressure (IMP) response to exogenous oxytocin after relaxin treatment is expressed as a percentage of the control amplitude.

relaxin at doses of 5 µg and 10 µg only affected the mammary response to exogenous oxytocin for a maximum of 18 min (Table 2), considerably shorter than the period during which reflex milk ejection was suppressed (Table 1). These results indicate that although relaxin also has an effect on the myoepithelium, the block of reflex milk ejection by all three doses of relaxin cannot be explained solely by the peripheral action of the hormone.

Our findings suggest that the inhibitory effect of relaxin on milk yield as reported by Knox and Griffith¹¹ in rats, and Cowie *et al.*¹² in goats, had a central component. The results also suggest that relaxin reduces *pre partum* myometrial activity by a double mechanism, that is, by a direct effect on the smooth muscle of the uterus and by a central action to prevent oxytocin release.

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Identity of the proliferating cell nuclear antigen and cyclin

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Studies of growth regulation and cellular transformation will be assisted by the identification of proteins that are preferentially synthesized in dividing cells. The 'proliferating cell nuclear antigen' (PCNA), distinguished by its apparent association with cell division, is defined by reaction with an antibody found in the autoimmune disease systemic lupus erythematosus (SLE)^{1,2}. This antibody reacts with proliferating cells including tumour cells but gives weak or undetectable immunofluorescence with resting cells of normal tissues^{2–6}. Peripheral blood lymphocytes are devoid of PCNA until activated by mitogen *in vitro*. In synchronized cultures its level and distribution fluctuate through the cell cycle, with a striking accumulation in the nucleolus late in the G₁ phase and early in the S phase³. Many of these properties are shared by 'cyclin'. This nuclear protein, identified by its position in a two-dimensional separation of cell proteins, is also transformation-sensitive and is preferentially synthesized in the S phase^{7–9}. We establish here that PCNA and cyclin are identical, and show that PCNA is an acidic nuclear protein of apparent molecular weight 35,000.

In a recent survey¹⁰, we found anti-PCNA antibodies in about 3% of SLE sera. Immunoglobulins prepared from nine sera of this specificity all precipitated a protein of molecular weight 35,000 from ³⁵S-methionine-labelled HeLa cell extracts (Fig. 1a), and we conclude that this protein is the antigen. No evidence was found for an associated RNA species, in contrast to the ribonucleoprotein antigens recognized by certain other antibodies in SLE, but weak labelling of PCNA with ³²P-phosphate suggested that it is at least partly phosphorylated (R.M.B.,

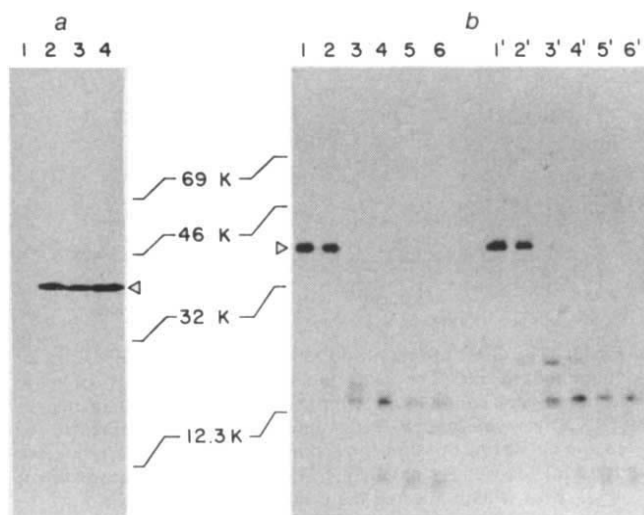


Fig. 1 Immunoprecipitation and peptide analysis of PCNA. *a*, Exponentially growing HeLa cell monolayers were labelled with ³⁵S-methionine for 5 h. Extracts were treated with control human IgG (lane 1) or with anti-PCNA IgG from each of three patients (lanes 2–4). Immune complexes were isolated using *S. aureus* as precipitant and were examined by electrophoresis in a 15% SDS-polyacrylamide gel as described elsewhere (ref. 16 and R.M.B., C. C. Bunn, G. R. V. Hughes, A. M. Francoeur and M.B.M., in preparation). *b*, Protein was extracted from gel bands containing immunoprecipitated PCNA (such as those in *a*) or from the two-dimensional gel spot identified as cyclin (as in Fig. 2*b*), by boiling in a solution containing 0.5% SDS, 10 mM 2-mercaptoethanol and 10 mM Tris-HCl pH 7.4. Aliquots of equal radioactivity were digested with protease from *S. aureus* strain V8 and examined in a 20% SDS-polyacrylamide gel. Lanes 1–6, immunoprecipitated PCNA incubated for 15 min at 37°C with 0, 0.1, 1, 10, 100 and 1,000 ng of V8 protease. Lanes 1'–6': two-dimensional gel-purified PCNA/cyclin (major spot) treated in the same way. Arrowheads indicate the PCNA band, and the positions of molecular weight markers are shown.

C. C. Bunn, G. R. V. Hughes, A. M. Francoeur and M.B.M., in preparation).

Examination of immunoprecipitates containing PCNA by two-dimensional gel electrophoresis (Fig. 2*a*) revealed a major spot with an apparent isoelectric point of 4.9 and MW ~35,000, together with a more acidic minor satellite spot. Removal of PCNA from the HeLa cell extract using anti-PCNA antibody led to the disappearance of both spots from the two-dimensional gel pattern (compare Fig. 2*b* and *c*), indicating that the spots contain PCNA only. Comparison of the HeLa cell protein pattern (Fig. 2*b*) with published gel patterns showed that PCNA resembles, in its apparent molecular weight and isoelectric point, the spot numbered IEF 49 and named 'cyclin' by Bravo and co-workers^{7,8}. The identity of cyclin with PCNA was confirmed by an exchange of samples between our laboratory and that of J. Celis (data not shown), and by construction of one-dimensional peptide maps¹⁰. The peptides released from immunoprecipitated PCNA by partial proteolysis with *Staphylococcus aureus* V8 protease (Fig. 1*b*) closely resembled those from the two-dimensional gel spot (Fig. 1*b'*) and were similar in size and distribution to those published for cyclin^{8,9}. A proteolysis pattern indistinguishable from that shown in Fig. 1*b'* was also obtained from the acidic satellite spot eluted from a two-dimensional gel (not shown).

PCNA is a prominent component of many tumour cell lines, including HeLa. To examine the biological properties of the protein, we used the REF52 line of rat embryo cells and its virus-transformed derivatives¹¹. Cultures of normal REF52 cells have a doubling time of 24 h and reach quiescence at a relatively low cell density, while Simian virus 40 (SV40)-transformed REF52 cells double in 18–19 h and reach a six- to eight-fold higher saturation density. PCNA was readily detected in a long-term labelled culture of proliferating REF52 cells (Fig. 3*a*),

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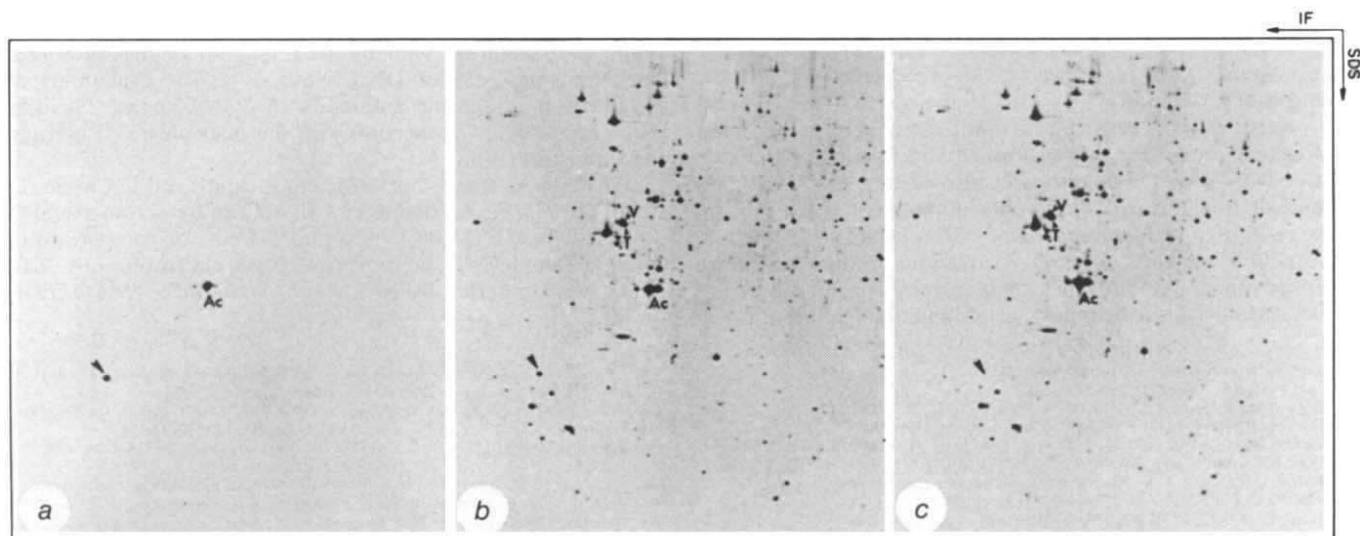
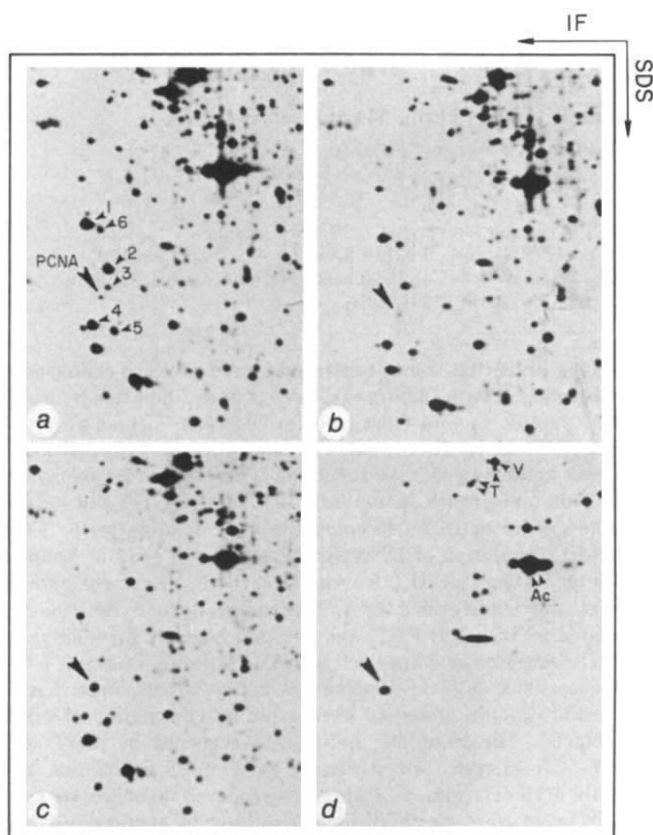


Fig. 2 Identification of PCNA in a two-dimensional gel. PCNA was precipitated from a HeLa cell extract as described in Fig. 1 legend. Proteins of the immunoprecipitate (a), the original extract (b) and the extract cleared of PCNA by anti-PCNA antibody (c), were resolved in two-dimensional gels using pH 3.5–10 LKB ampholytes in the first dimension (IF) and 10% acrylamide/SDS in the second dimension (SDS). Gels b and c were loaded with 500,000 d.p.m. of labelled protein and exposed for 9 days; gel a was loaded with 5,800 d.p.m. of labelled protein and exposed for 7 days. All gels shown were processed for fluorography¹⁷ and exposed to XS-1 film. Arrowheads indicate the position of the major PCNA spot. The actin (Ac), vimentin (V) and tubulin (T) spots are also indicated. Actin is precipitated nonspecifically by both anti-PCNA and control sera, in amounts that vary between experiments. The gel shown in a contains actin serving as internal marker.

Fig. 3 (Right) PCNA in normal and transformed rat cells and purified nuclei. Proteins of proliferating REF52 cells 3 days after plating (a), quiescent REF52 cells 8 days after plating (b) and dividing REF52-AG6 cells 3 days after plating (c) were labelled for 24 h with ³⁵S-methionine and resolved in two-dimensional gels as in Fig. 2. Nuclear proteins from REF52-WT6 cells labelled for 2 h with ³⁵S-methionine were resolved in a similar gel (d). Nuclei were prepared by the method of Fleischer and Kervina¹⁸. REF52-WT6 cells are SV40-transformed REF52 cells¹¹, and REF52-AG6 cells are REF52-WT6 cells selected for growth in soft agar. Approximately 300,000 d.p.m. of labelled protein were applied to each gel. Gels a–c were exposed for 17 days and gel d for 4 days. The proteins indicated are the major PCNA spot (large arrowheads), actin (Ac), vimentin (V), tubulin (T) and tropomyosins 1–6 (numbers in a). The tropomyosin forms and their changes on transformation have been described elsewhere^{12,19,20}. Quantitation was carried out using the QUEST system for computerized analysis of two-dimensional gels²¹. Each film was scanned using an Optronics P-1000 densitometer, and the spots were detected and integrated by the image analysis programs. Each film was calibrated²² so that measurements of film density could be converted to d.p.m. per unit area before spot integration. The integrated d.p.m. for each spot was divided by the total trichloroacetic acid-precipitable d.p.m. applied to the gel. For the protein samples resolved in a–c, the proportion of total applied d.p.m. found in the PCNA spots was 0.014%, 0.008% and 0.067%, respectively.

but it was not a major protein constituent. It was also detectable in a resting culture of normal REF52 cells (Fig. 3b), although its level at quiescence was about half that in dividing cells. On the other hand, subconfluent SV40-transformed cells (Fig. 3c) contained about fivefold more PCNA than normal dividing REF52 cells (Fig. 3a). The magnitude of this increase, one of the largest inductions seen on transformation of REF52 cells by SV40 (data not shown), makes it unlikely that the higher level of PCNA in the transformed cells is due simply to their slightly shorter generation time. Furthermore, pulse-labelling experiments show that the rate of PCNA synthesis is elevated in all transformed lines examined, including cells transformed by SV40, adenovirus type 5 and Kirsten murine sarcoma virus¹².

In both HeLa cells (not shown) and SV40-transformed REF52 cells (Fig. 3d), PCNA was a major component of the nuclear fraction. It was more prominent in the nuclear pattern than in the whole cell protein pattern (Fig. 3c), unlike cytoplasmic components such as the tubulins and vimentin which were detectable but not enriched in the nuclei. However, the enrichment of PCNA in the nuclear fraction was not as great as that



seen for certain other nuclear proteins. Indeed, some PCNA was detected in the cytoplasmic fraction of these cells (not shown), but in view of the overwhelmingly nuclear staining seen by immunofluorescence^{2,3} this may represent a partial leaching from the nucleus during cell fractionation rather than a distribution between the two cellular compartments *in vivo*. Like its human counterpart, the rat PCNA spot was accompanied by a more acidic satellite spot. The ratio of the two forms did not vary substantially as a function of growth rate or subcellular fractionation, and the significance of this minor form is unclear.

These results demonstrate that PCNA is identical with cyclin in subcellular localization, electrophoretic behaviour and

peptide composition, as well as in biological properties. We suggest that the name PCNA be used in future, as it is the earlier nomenclature and the term cyclin has recently become ambiguous¹³. As shown here and elsewhere^{3-9,12}, the level of the protein falls in resting cells and, conversely, it becomes particularly prominent in transformed and tumour cells. Fluctuating PCNA levels have probably also been observed in other studies of transformed¹⁴ and serum-stimulated¹⁵ cells, but in these cases definite identification of PCNA is lacking. Although its function remains obscure, correlation of the previously unlinked reports on this transformation-sensitive and cell cycle-associated nuclear protein may yield illuminating clues. In this

regard, it is intriguing that the maximal synthesis of PCNA appears to coincide with its accumulation in the nucleolus, preceding the peak of DNA synthesis^{3,7}. The availability of relatively monospecific antibodies to PCNA should facilitate exploration of its connection with the nucleolus, cell division and transformation.

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Synthesis of biologically active rat transforming growth factor I

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The hypothesis that some transformed cells produce endogenous transforming growth factors (TGFs) has been supported by isolation of peptide factors from transformed cells¹⁻⁵. One group of TGFs, TGF-I, produced only by transformed cells, displays sequence homology with the functionally related mouse submaxillary epidermal growth factor (mEGF)⁵. Both TGF-I and mEGF exhibit similar activities in competition for binding to the EGF receptor, stimulation of DNA synthesis and cell growth. Another group of TGFs, TGF-II (also known as TGF β)², present both in normal and transformed cells, is structurally and functionally unrelated to TGF-I or EGF, and does not compete for binding to the EGF receptor or induce cell growth. However, TGF-I or EGF in the presence of TGF-II produces a synergistic effect that is responsible for the observed phenotypic transformation of NRK fibroblasts⁴. The complete amino acid sequence of rat TGF-I (rTGF-I) from transformed Fischer rat embryo fibroblasts, has recently been determined⁵. Using this proposed sequence, we have now prepared synthetic rTGF-I by the solid-phase synthesis method and find that it exhibits chemical and biological properties indistinguishable from those of natural rTGF-I. Since synthetic rTGF-I is free of any biological contaminants, our findings provide independent evidence that rTGF-I is an active principle in the transformation of cells⁶.

Rat TGF-I purified from the conditioned medium of Fisher rat embryo fibroblasts transformed by feline sarcoma virus is a single peptide chain containing 50 amino acid residues and 3 disulphide linkages. The primary structure was determined by Edman degradation in a gas-phase microsequencer to be: H-Val-Val-Ser-His-Phe-Asn-Lys-Cys-Pro-Asp-Ser-His-Thr-

Gln-Tyr-Cys-Phe-His-Gly-Thr-Cys-Arg-Phe-Leu-Val-Gln-Glu-Glu-Lys-Pro-Ala-Cys-Val-Cys-His-Ser-Gly-Tyr-Val-Gly-Val-Arg-Cys-Glu-His-Ala-Asp-Leu-Leu-Ala-OH.

The chemical synthesis of rTGF-I was performed manually by the stepwise solid-phase approach, following the general principles described by Merrifield⁷. We adopted the differential acid-labile protecting group strategy, using the conventional combination of t-butyloxycarbonyl for the N α -amino terminus and benzyl alcohol derivatives for the side chains. An improved, more acid-stable benzyl ester linkage that anchored protected amino acids to the polymeric support was used to minimize loss of peptides during the repeated acid treatments⁸. Complete deprotection and removal of peptide from the resin was achieved by the new low-high HF method^{9,10}, which removed benzyl protecting groups by the S_N2 mechanism in dilute HF solution; this minimizes the serious side reactions due to carbocations generated in the conventional S_N1 deprotection method. It also minimizes the cysteinyl side reactions that can hamper the synthesis of proteins containing multiple disulphide linkages.

After HF treatment and before any purification, the crude and reduced synthetic rTGF-I was oxidized and regenerated by the mixed disulphide method in the presence of a combination of reduced and oxidized glutathione¹¹. This avoided the formation of polymeric materials during purification. The regenerated, crude rTGF-I contained 40-50% of EGF radioreceptor and tyrosine-specific protein kinase activities when compared with natural rTGF-I. Crude synthetic rTGF-I was purified to homogeneity in three steps: (1) gel filtration on a BioGel P-10 column, (2) ion-exchange chromatography on a CM-Sephadex column and (3) preparative HPLC on a C₁₈ reverse-phase column. Overall yield, based on starting loading of Ala to resin, was 31%.

In both reducing and nonreducing conditions, SDS-polyacrylamide gel electrophoresis of the purified synthetic rTGF-I gave a single band with an apparent molecular weight of 7,000. Amino acid analysis by 6M HCl and enzymatic hydrolysis provided the expected theoretical molar ratio of the proposed sequence. No free thiol was detected in synthetic rTGF-I by Ellman's method of sulphhydryl determination¹², but thiolytic reduction produced the expected theoretical value of six cysteine residues. These findings support the conclusion that synthetic rTGF-I is a single-chain polypeptide containing six cysteines in disulphide linkages, which agrees with the expected chemical properties of natural rTGF-I. More importantly, synthetic rTGF-I co-eluted

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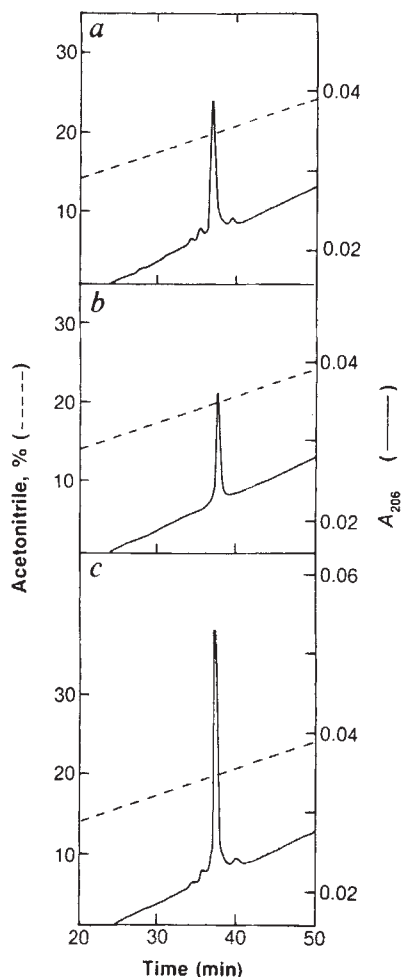


Fig. 1 HPLC of synthetic and natural rat TGF-I. *a*, 33.3 EGF-equivalents of natural rTGF-I; *b*, 26.3 EGF-equivalents of synthetic rTGF-I; *c*, 33.3 EGF-equivalents of natural rTGF-I and 26.3 EGF-equivalents of synthetic rTGF-I. Unit EGF equivalency is based on the displacement of ^{125}I -EGF in the radioreceptor assay.

Methods: Crude synthetic rTGF-I was batch purified by gel filtration (P-10 column, 2.5×75 cm, elution with 1 M acetic acid), ion-exchange chromatography (CM-Sephadex column, 2.5×15 cm, linear pH gradient of 5.6–8.0 in 0.1 M NH_4OAc) and finally preparative HPLC (2.5×30 cm at a flow rate of 1.4 ml min^{-1} , eluting with a linear gradient of acetonitrile in 0.033% trifluoroacetic acid). Analytical HPLC was performed on a $\mu\text{Bondapak C}_{18}$ column ($10 \mu\text{m}$ particle size, 0.39×30 cm, Waters Associates) at a flow rate of 1 ml min^{-1} at 40°C . Peptides were eluted with a linear gradient of acetonitrile in 0.045% trifluoroacetic acid and were monitored at 206 nm.

with natural rTGF-I as a single symmetrical peak in C_{18} reverse-phase HPLC (Fig. 1).

Synthetic rTGF-I was compared with natural rTGF-I in three assays for the biological properties of the putative transforming growth factor. In the mitogen assay, TGF-I-stimulated growth of serum-deprived normal rat kidney cells was measured by the incorporation of ^{125}I -iododeoxyuridine. The soft agar assay, performed in the presence of fetal bovine serum and TGF-II, confirmed that the morphological and phenotypic alterations induced by rTGF-I could be quantified by colony formation in soft agar. The latter assay has been shown to correlate well with tumorigenicity¹³. Fetal bovine serum or TGF-II alone does not induce transformation of NRK cells in culture. Similarly, neither natural nor synthetic TGF-I produces such an effect in the absence of TGF-II. Both synthetic and natural rTGF-I displayed similar dose-response curves and half-maximal activities in these two assays (Fig. 2).

As rTGF-I is structurally related to EGF^{14,15} and competes with mEGF for the binding of EGF receptors on cellular mem-

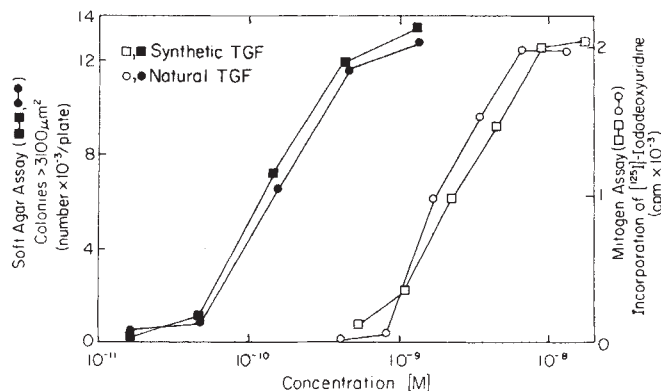


Fig. 2 Dose-response curves of mitogen and soft agar growth assays of synthetic and natural rTGF-I on normal rat kidney cells. The mitogen assay (open circles and squares) was performed in flat-bottom microwells (1×10^4 cells per well) in 100 μl Dulbecco's minimal essential medium with 10% heat-inactivated fetal bovine serum for 24 h and maintained in medium containing 0.1% fetal bovine serum for 7 days before the assay. Test samples were kept in 50 μl Waymouth medium¹⁹, and $[5\text{-}^{125}\text{I}]\text{iodo-2'-deoxyuridine}$ (^{125}I -IUDR, $2.5 \mu\text{Ci ml}^{-1}$, Amersham) was added for the final 24 h of culture²⁰. Cells were washed and collected using a multiple harvester and counted in a γ -counter. Maximal ^{125}I -IUDR incorporation (144 c.p.m.) was 14-fold above control values (14 c.p.m.) without added mitogen. Values were corrected by subtracting ^{125}I -IUDR incorporated into unstimulated control cultures. The assay for colony growth in soft agar (solid circles and squares)²¹ using 49F fibroblast cells was performed as reported elsewhere²², except that the crude platelet-derived murine TGF-II was added from stocks of 1 mg ml^{-1} in phosphate-buffered saline, containing 1 mg ml^{-1} bovine serum albumin. Stained colonies were quantified by number and size using a Bausch and Lomb image analyser²³. The number of soft agar colonies ($> 3,100 \mu\text{m}^2$) in the presence of TGF-II was 96 per plate and was subtracted.

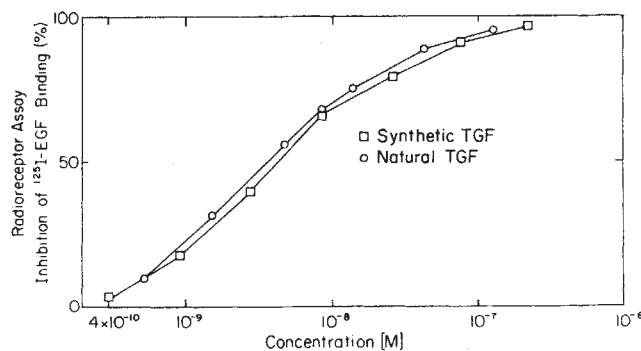


Fig. 3 Dose-response curve of radioreceptor assay of synthetic and natural rTGF-I. Purified mEGF was radiolabelled with $\text{Na } ^{125}\text{I}$ by a modified chloramine-T method as described previously⁵. The ^{125}I -mEGF binding assay¹ was performed on subconfluent monolayers of formalin-fixed A431 cells¹. Cells (8×10^3 per dish) were incubated with ^{125}I -mEGF (0.8 ng ml^{-1} , $82 \mu\text{Ci per } \mu\text{g}$) and unlabelled peptides at the indicated concentrations. The concentrations of natural and synthetic rTGF were quantified by amino acid analysis of companion aliquots. The ^{125}I -mEGF bound was determined, and nonspecific binding (binding in the presence of $2 \mu\text{g ml}^{-1}$ mEGF) was 2.4% and was subtracted. 100% binding corresponded to 10% of the input radioactivity.

branes¹, synthetic rTGF-I was compared with natural rTGF-I for binding on A431 human carcinoma cells. Again, the response and activities of the natural and synthetic rTGF-I were indistinguishable from each other (Fig. 3). The concentration required for 50% inhibition of ^{125}I -EGF binding was 3.5 and 4.1 nM for natural and synthetic rTGF-I, respectively. A consequence of TGF or EGF binding to the EGF membrane receptors is the stimulation of phosphorylation of tyrosine residues in synthetic peptides or endogenous substrates^{16,17}. Synthetic rTGF-I was found to stimulate the phosphorylation of the synthetic angiotensinyl peptide substrate¹⁸ with a half-maximal activity

of 0.3 nM (data not shown), comparable with the reported activity for natural rTGF-I¹⁶.

In conclusion, we have demonstrated that synthetic rTGF-I possesses chemical and biological properties indistinguishable from those of natural rTGF-I. These findings, therefore, provide convincing evidence that the chemical structure of rat TGF-I is that proposed by sequencing studies⁵. Since synthetic rTGF-I is completely free of biological contaminants, its biological properties must be entirely due to the proposed structure of rat TGF-I, confirming the view that transformed cells produce endogenous growth stimulating polypeptide factors.

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Reversible inhibition of translation by *Xenopus* oocyte-specific proteins

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A characteristic of growing oocytes of all animal species is the synthesis and accumulation of messenger RNA which is destined to be used primarily by the early embryo^{1,2}. The mechanism(s) which regulates the translation of this maternal mRNA remains unknown. However, the inability of the oocyte to translate all of its putative mRNA has been attributed to at least three limitations: (1) The rate of translation is limited by the availability of components of the translational apparatus other than mRNA^{3,4}, (2) the structural organization of the mRNA prevents translation^{5,6}, and (3) proteins associated with the mRNA prevent translation^{7,8}. Several investigators have suggested that proteins associated with maternal mRNA suppress translation in sea urchin eggs^{7–10}, although others claim that such results may be due to experimental artefacts^{11,12}. Oocyte-specific proteins have been identified in association with non-translating poly(A)⁺ mRNAs from *Xenopus laevis* oocytes^{13–16}, and we report here that when these proteins are reconstituted with mRNAs *in vitro* the translation of the mRNAs *in vitro* is reversibly repressed. The implication is that these proteins are involved in the regulation of translation of stored maternal mRNAs.

Recent studies have demonstrated that RNAs and proteins may be reconstituted faithfully *in vitro* (for example, refs 17–19).

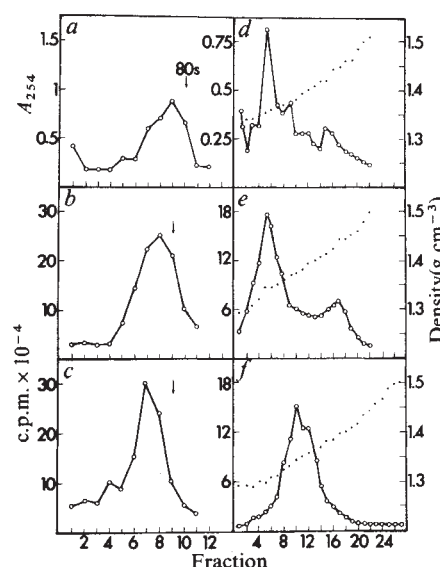


Fig. 1 Centrifugation of native and reconstituted RNPs. Formaldehyde-fixed native RNPs from oocytes were centrifuged through a sucrose gradient, fractionated and the absorbance at 254 nm in each fraction was determined (a). The fractions corresponding to 40–60S (fractions 8, 9) were collected, dialysed and centrifuged through a CsCl gradient. Fractions (0.2 ml) were collected and absorbance at 254 nm was determined, as was the density which was calculated from refractive index (d). Iodinated poly(A) RNA from stage 1–2 oocytes and rabbit globin mRNA were reconstituted with the RNA binding proteins and were centrifuged through sucrose gradients (poly(A) RNP (b); globin mRNA (c)). The 40–60S fractions from these gradients were then collected, fixed and centrifuged on CsCl gradients (poly(A) RNP (e); globin RNP (f)). Values for radioactivity (○—○) and density (·····) are shown. The arrow indicates where the 80S monosome sedimented.

Methods: Stage 1–2 *Xenopus* oocytes were homogenized in poly-some buffer (0.3 M KCl, 2 mM MgCl₂, 20 mM Tris-HCl pH 7.4, 4 μg ml⁻¹ polyvinyl sulphate, 0.01% diethylpyrocatechol, 10 μg ml⁻¹ cycloheximide and 0.5% Nonidet P-40) and the post-mitochondrial supernatant was then centrifuged through a 5–30% sucrose gradient in buffer in an SW41 tube for 3 h at 4 °C at 34,000 r.p.m. The gradient fractions corresponding to the 40–60S region were collected, dialysed against 10 mM phosphate buffer pH 7.2, and then fixed in phosphate-buffered 4% formaldehyde for 2 h at 4 °C²⁷. These particles then were suspended in 10 mM HEPES pH 7.5 and 0.5 M NaCl and applied to an oligo-d(T) cellulose column (P-L Biochemicals, type 7). The column was washed in high salt buffer until no UV(A₂₅₄)-absorbing material was detected in the eluate. The poly(A) RNPs were then eluted in 10 mM HEPES pH 7.5. Fixed RNPs were centrifuged through preformed 30–60% CsCl gradients²⁸ for 12 h in an SW 50.1 tube at 33,000 r.p.m. at 4 °C. Poly(A) RNA and globin mRNA were iodinated by the ¹²⁵I method as described previously^{6,16}.

To obtain oocyte protein for reconstitution experiments, stage 1–2 *Xenopus* oocytes were homogenized and the ribonucleoprotein (RNP) particles sedimenting through a sucrose gradient to a 40–60S fraction were collected. The proteins from such RNPs were purified further by elution from a phosphocellulose column in high salt as reported previously¹⁶. The five major proteins in this fraction have molecular weights of 50,000–94,000, bind poly(A)⁺ RNA with high affinity and are oocyte-specific¹⁶. For reconstitution studies, these proteins were mixed with ¹²⁵I-labelled poly(A) RNA from stage 1–2 oocytes and compared with native particles by sucrose gradient and CsCl gradient centrifugation. Reconstituted poly(A) RNP particles sedimented primarily at 40–60S (Fig. 1b), as did the native particles originally used to obtain the proteins used for reconstitution (Fig. 1a). Most of the reconstituted poly(A) RNP particles which sedimented at 40–60S (Fig. 1b) exhibited a buoyant density in CsCl of 1.34 g cm⁻³, with a minor peak at a density of 1.44 g cm⁻³ (Fig. 1e). These values again are virtually identical with those obtained with native particles which exhibited a peak

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Table 1 Relative translatability of reconstituted globin mRNA in injected oocytes

	Synthesis of globin relative to injected pure globin mRNA (%)						x $\pm$ s.d.
	1	2	3	Expt no. 4	5	6	
Pure globin mRNA	100	100	100	100	100	100	100 $\pm$ 0
Globin mRNA + histones	140	139	133	111	—	—	131 $\pm$ 12
Globin mRNA + ribosomal proteins	—	150	155	120	—	—	142 $\pm$ 15
Globin mRNA + basic liver proteins	—	105	137	101	—	—	114 $\pm$ 16
Globin mRNA + basic leg muscle proteins	—	—	—	—	—	97	97
Globin mRNA + basic heart proteins	—	—	—	—	—	96	96
Globin mRNA + oocyte RNA binding proteins	5	30	37	11	40	37	25 $\pm$ 17

Expt 1: Four stage VI *Xenopus* oocytes were injected with 10 ng of pure globin mRNA, 10 ng of globin mRNA reconstituted with 10 ng calf thymus histones, or 10 ng of oocyte binding proteins (preparation 1) prepared as follows. Stage 1–2 *Xenopus* oocytes were homogenized and the post-mitochondrial supernatant was centrifuged through a sucrose gradient¹⁶. The RNPs sedimenting to the 40–60S fraction were collected and applied to a phosphocellulose column. The proteins eluting in 1–2 M NaCl were used for reconstitution¹⁶. Reconstitution conditions were 30–60 min on ice in 10 mM Tris-HCl pH 7.5 and 50 mM NaCl. Oocytes were cultured for 12 h in OR-2 medium²⁴ and then injected with ³H-leucine⁴. Oocytes were then cultured for 1 h. The radioactive proteins were collected⁴, electrophoresed on a SDS-12.5% polyacrylamide gel and the radioactive globin band was cut out, oxidized and the radioactivity determined by liquid scintillation spectrometry⁴. **Expts 2–6:** Five oocytes were injected with 3 ng pure globin mRNA, or globin mRNA reconstituted with 30 ng of calf thymus histones, *Xenopus* oocyte ribosomal proteins (that is, *Xenopus* stage VI oocyte proteins extracted from RNP particles with high salt (2 M NaCl) which sedimented at 80S and had a molecular weight <60,000 as determined by gel filtration), *Xenopus* liver, leg muscle and heart proteins prepared in the same way as the oocyte RNA binding proteins¹⁶, or *Xenopus* stage 1–2 oocyte RNA binding proteins¹⁶. Oocytes were cultured for 18 h in modified Barth's saline²⁵ and then injected with ³⁵S-methionine²⁶. Oocytes were cultured for an additional hour and the protein prepared as described previously⁴. The relative amount of globin synthesis was determined by densitometry of autoradiograms or by direct quantitation of the radioactivity in the putative globin band. Expt 2 used oocyte RNA binding protein preparation number 2, expt 3 used preparation 3, expt 4 used preparation 4 and expt 5 used preparation 5. The autoradiogram shown in Fig. 2 was taken from expt 4.

density of 1.36 g cm⁻³, with a minor peak at 1.42 g cm⁻³ (Fig. 1d). Similar experiments were performed with ¹²⁵I-labelled rabbit globin mRNA. Reconstituted globin mRNA also sedimented in the 40–60S region of the gradient (Fig. 1c) and had a peak buoyant density of 1.34 g cm⁻³ (Fig. 1f). Using the formula of Spirin²⁰, the native RNPs had a protein to RNA mass ratio of 4.5:1, very similar to a previous estimate of oocyte poly(A) RNP protein to RNA mass ratio of 4:1 (ref. 14). The reconstituted poly(A) RNP and globin RNP had protein to RNA mass ratios of 5.5:1. Other experiments (data not shown) demonstrated that that same ratio was obtained even with a 10-fold greater amount of protein in the initial incubation with the RNA. Thus, based on the physical characteristic we have measured, the reconstituted RNPs appear to be very similar to native RNPs.

To test the translatability of reconstituted RNPs *in vitro*, we have injected RNPs into *Xenopus* oocytes. Since the endogenous mRNAs being translated represent a subset of total poly(A) RNA in the oocyte, the translation of reconstituted poly(A) RNAs would be difficult to assess quantitatively⁶. Accordingly, we have determined whether the oocyte-specific RNA binding proteins affect the translation of globin mRNA. Figure 2 shows an SDS gel autoradiogram of the proteins synthesized in mRNP-injected oocytes. When pure globin mRNA was injected, globin synthesis was readily detected (Fig. 2, lane 2). However, when globin mRNA was reconstituted with oocyte proteins before injection, no globin synthesis was apparent (lane 3). Globin mRNA reconstituted with other basic proteins such as histone (lane 4) or ribosomal proteins (lane 5) was still translatable. Furthermore, reconstitution of globin mRNA with basic liver proteins (lane 6), extracted from the 40–60S particle fraction and purified as described for the oocyte proteins, did not prevent translation.

Table 1 summarizes several experiments to determine the amount of globin synthesized as a consequence of reconstitution with protein from several sources. The reconstitution of globin mRNA with histones or ribosomal proteins or proteins from frog liver, muscle or heart prepared in a manner identical with the oocyte proteins described above, results in as much as 42% enhancement of globin synthesis. Examination of the protein species in these various fractions reveals that those present in the oocyte are absent from the somatic tissues (data not shown; see also ref. 16). As most globin mRNA turns over following



Fig. 2 Autoradiogram of proteins synthesized in mRNP-injected oocytes. Oocytes were injected with 5 ng of pure rabbit globin mRNA (lane 2), or 5 ng globin mRNA reconstituted with 30 ng of stage 1–2 oocyte proteins (lane 3), 30 ng of histones (lane 4), 30 ng of ribosomal proteins (lane 5), or 30 ng basic liver proteins (lane 6). Control oocytes were not injected (lane 1).

Methods: Conditions for reconstitution are described in Table 1 legend. Oocytes were injected with mRNA or mRNP, cultured for 18 h, injected with ³⁵S-methionine (Amersham; 1,220 Ci mmol⁻¹) and cultured for an additional hour. Oocytes were homogenized in 50 mM NaCl, the protein was collected⁴, and resolved by SDS-12.5% polyacrylamide gel electrophoresis.

injection⁴, it is likely that the non-oocyte proteins protect the mRNA from oocyte nucleases until it is loaded onto polysomes. As Fig. 2 indicates, the oocyte-specific proteins inhibit globin synthesis in injected oocytes. On average, one-quarter as much globin is synthesized when the mRNA is reconstituted with the oocyte proteins as when pure mRNA is injected. Although there was some variation between preparations, the protein always inhibited globin mRNA translation by at least 60%.

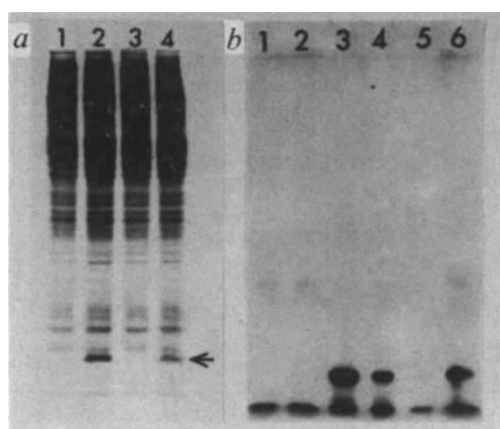


Fig. 3 The inhibition of translation by oocyte-specific proteins is reversible. *a*, Five noninjected oocytes (lane 1), or oocytes injected with 5 ng pure globin mRNA (lane 2), 5 ng globin mRNA reconstituted with oocyte proteins (lane 3), or 5 ng globin mRNA reconstituted with oocyte proteins and then deproteinized (lane 4), were cultured and then injected with ³⁵S-methionine. The resulting radioactive proteins were resolved by SDS-gel electrophoresis and fluorography. *b*, Synthesis of reconstituted globin mRNA in a wheat germ lysate. Lane 1, no mRNA added; lane 2, 1 µg globin mRNA reconstituted with 1 µg of oocyte proteins and then digested with 1 µg ml⁻¹ RNase A; lane 3, 1 µg pure globin mRNA; lane 4, 1 µg globin mRNA reconstituted with 1 µg oocyte protein; lane 5, 1 µg globin mRNA reconstituted with 9 µg oocyte protein; lane 6, 1 µg globin mRNA reconstituted with 9 µg oocyte protein and then deproteinized.

Methods: Globin mRNA and oocyte proteins were reconstituted as described in Table 1 legend (expt 4). One-half of the mixture (as well as pure RNA) was extracted once with buffer (10 mM Tris-HCl pH, 0.1% SDS)-saturated phenol/chloroform/isoamyl alcohol (25:24:1) and once with chloroform/isoamyl alcohol (99:1). The aqueous phases were then injected without further modification. The efficiency of recovery was ~80% based on the extraction of reconstituted ¹²⁵I-globin mRNA. Following RNP or RNA injection, oocytes were cultured for 18 h and then injected with ³⁵S-methionine. The resulting radioactive proteins were collected and analysed as described for Fig. 2. The wheat germ lysate (BRL) reaction volume was 30 µl containing 5 µCi of ³⁵S-methionine. The reaction was carried out at 25 °C for 1 h. Deproteinization and the analysis of the radioactive proteins were as described above.

We have assayed for ribonuclease contamination in the oocyte protein preparation. ³²P-labelled total oocyte RNA was mixed with the oocyte proteins and the RNA was resolved by denaturing agarose gel electrophoresis and autoradiography. In contrast to pure ³²P-labelled oocyte RNA, no degradation of the reconstituted RNA was observed (data not shown). In addition, total RNA from oocytes injected with globin mRNA reconstituted with the oocyte proteins was extracted and used for an RNA gel blot. Using ³²P-labelled globin cDNA as the probe, only full-size globin mRNA was evident when pure or reconstituted globin mRNA was injected (data not shown). However, the possibility remained that the oocyte proteins rendered the mRNA nontranslatable by some permanent modification not detected by the methods described above. Therefore, we determined whether reconstituted globin mRNA may become translatable following deproteinization. Figure 3a again demonstrates that globin synthesis was detected when pure mRNA (lane 2), but not reconstituted mRNA (lane 3), was injected into oocytes. When reconstituted mRNA was deproteinized by phenol and chloroform and then injected, globin synthesis was evident (lane 4). The synthesis of globin in oocytes injected with deproteinized reconstituted globin mRNA was always 20–40% lower than when the pure mRNA was injected. This difference cannot be accounted for by a very low recovery of the mRNA

(see Fig. 3 legend) and possibly results from incomplete deproteinization. Nonetheless, we do conclude that the oocyte proteins reversibly inhibit the translation of injected mRNA in injected oocytes.

The ability of the oocyte proteins to inhibit translation of globin mRNA *in vitro* was also tested (Fig. 3b). Whereas the translation of globin mRNA was very efficient in a wheat germ lysate (lane 3), that of mRNA reconstituted with increasing amounts of the oocyte proteins became progressively more inefficient (lanes 4, 5).

We have conducted preliminary experiments which suggest that mRNP unmasking can occur *in vivo*. These experiments are based on the observation that in oocytes induced to undergo maturation by progesterone, general protein synthesis approximately doubles²¹ due to the recruitment of previously untranslated mRNA onto polysomes²². If this recruitment involves mRNP unmasking, it might be expected that injected globin mRNP would become translatable in the mature oocyte. When such experiments were done in oocytes injected with reconstituted globin mRNP and then induced to mature, there was a 3.5-fold increase in radioactivity in the globin band. However, in noninjected oocytes which had undergone maturation, a 2.9-fold increase in radioactivity occurred in the region of an SDS gel where globin would be expected to migrate. Although we tentatively attribute this difference to globin mRNP unmasking, the fact that the synthesis of endogenous proteins partially obscures our quantitation of globin synthesis suggests the need for a more rigorous study to confirm these observations.

The present study demonstrates that oocyte-specific proteins extractable from native RNPs may be reconstituted with mRNAs *in vitro* to form particles which resemble native RNPs. Further, these proteins can reversibly repress the translation of the mRNA *in vivo* (as well as *in vitro*). Several control experiments support the view that this result is not due to nonspecific inhibition. Thus, we now have a system whereby proteins can be used in reconstitution experiments and their ability to alter translation and/or stability of mRNA in *in vivo* conditions may be investigated. In this connection, a monoclonal antibody to an oocyte RNA binding protein has been isolated²³. Thus, with this and other monoclonal antibodies, individual proteins may be isolated and reconstituted with mRNA, and the function of each assessed.

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French change

Guy Ourisson

Pour Sauver l'Université. By Laurent Schwartz.

Editions du Seuil, 27 rue Jacob, F-75261, Paris Cedex 06: 1983. Pp.123. Pbk FF 49.

LAURENT Schwartz, a Fields medallist, is certainly the most outspoken mathematician in France. For years, he has persistently taken strong public stands against colonialism in Algeria, for human rights and civic liberties in the USSR, for progress through education and scientific research, and for the electoral victory of the Left in France. So when he writes of the reforms currently underway in French universities that "mediocrity will take precedence over quality, diversity will disappear into drabness, responsible decisions will be made impossible by irresponsible politicking", everybody in French academic circles is likely to listen to what he has to say.

Schwartz sets the tone unambiguously: "I hope the present socialist experiment will succeed. I want to help the Government, and this is why I criticize it". Despite this clear warning, I had expected, when this book appeared last autumn, that his analysis — expert, explicit and caustic — would become political TNT graciously handed to the opposition parties. This, in fact, has not really been the case; when the book came out, the opposition had already shifted its attention to more popular disputes than the Savary reform. Such is the prodigious capacity of Gallic organizations to lose interest once a law has been passed and all that remains to do is to try to apply it.

Pour Sauver l'Université has now been read by everybody in French academic

circles; at least, the reviews have been read. The book has helped to focus the final round of discussions in Parliament on some of the key points of the Savary reform. It has helped in generating, in the universities, new trends and new view points. It has been a useful book, but one which has in part estranged Schwartz's own Left, the author being branded as an elitist, an arch-enemy of the Unions and the avowed leader of the "mandarins" (in France, the reactionary professors). Schwartz's influence has rallied around the slogan "Qualité de la science" lists of candidates, politically non-aligned and independent of the trade unions, in recent elections to various national "Comités" — and his name has helped these candidates to be elected *en masse*. So the impact of this little book in France has been great. But who outside of France would discover it to be worth reading?

What is found in *Pour Sauver l'Université* ranges from the specific to the general. I doubt if anybody outside France, except perhaps students of French education, would bear reading the chapter criticizing the new voting procedures chosen for the various bodies of university self-government (those same procedures which a few months later enabled "Qualité de la science" candidates to be elected in great numbers). I also doubt if anybody outside the French system would gain anything useful from the chapter describing the dual higher-education system in

France, in which Grandes Ecoles thrive alongside universities, select supposedly the best students, in some areas monopolize the job market, nurture the industrial elite, and yet are in many cases appallingly alien to the very notions of innovation and of research. We French have to cope with this system, and try to adapt it to changing needs — but nobody else would gain from adopting it.

On the other hand, it is quite probable that a longer lasting interest could be found in the three remaining chapters: two on the virtues of selection of students, and on the way to organize it, and the last one on the interaction between teaching and research. These are real, general problems, common to many countries, and Schwartz has a very wide knowledge of the situation in the United States, in Great Britain and in Germany. His opinions therefore carry some weight. For instance, he gives a very lucid discussion of the fallacy equating free entry into universities with the democratization of higher education — particularly when students, once admitted, are not orientated towards fields adapted to their skills and thus in part towards the job market, but have to cope unassisted with examiners determined to let only the fittest survive. He also broadly defines conditions which would favour a genuine democratization of the higher education system — by setting ambitious goals for students and helping them to choose progressively the area in which they could excel, or at least do their best. Admittedly, this is the very philosophy of the Savary reform; but here, as in many other places in the book, Schwartz is more critical of what he fears will be made of these changes, by us his colleagues, than of the content of the new law itself. Obviously, he shares with me a certain scepticism of the real efficiency, in France, of a "definitive" law.



Students march through Paris in May last year in protest at the proposed reform of the nation's university system.

For foreign readers, however, probably the most informative chapter is the one devoted to research (more precisely, to remain in tune with the book's general theme, to the "Dangers Looming over Research"). This gives a good description of what really works well in French research (apart from Big Science): the unique symbiosis of the National Research Council (the Centre National de la Recherche Scientifique) and the universities, which might well be a worthwhile export item. Schwartz believes it is endangered; I do not, and I do not believe that any law can threaten it more than our natural tendency to divide Gallia into as many little fiefdoms as we can.

Anybody interested in understanding the currents now flowing in French academia, and shaping it anew, should read this short and intelligent book. A complementary audience might also include students of academic research as well as some sociologists and historians. But whatever their background, all readers should remember to place Schwartz's thesis within a general perspective of the French political situation in which it is rooted. □

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ADVERTISEMENT

The current issue of *Peptides*: vol 5, no. 2 (March/April), 1984, is devoted to recent progress in research on vaso-active intestinal polypeptide (VIP) and related peptides. The papers, based on presentations at the 1st international symposium on VIP, held in Brussels, September 13–16, 1983, deal with aspects of chemistry, biochemistry, anatomic localization, physiology, pathophysiology and clinical significance of VIP (and to some extent, secretin & PHI). Topics relate to the neuroendocrine, gastrointestinal, cardiovascular, respiratory, and reproductive systems.

The journal may be ordered from: ANKHO International, Inc., P.O. Box 426, Fayetteville, NY 13066

Underwater illumination

M. J. Dring

Light and Photosynthesis in Aquatic Ecosystems.

By John T. O. Kirk.

Cambridge University Press: 1983.

Pp.401. £37.50, \$74.50.

THERE are advantages and disadvantages in attempting to bridge the gap between two scientific subjects. Ideally, the bridge will provide a route for the two-way traffic of ideas and scientists; but if the two subjects are in very different states of development, it may result instead in a brain-drain from the weaker to the stronger subject.

By bringing together the physics of underwater light and the biology of aquatic photosynthesis, Dr Kirk's book seems more likely to produce the latter result. Part I ("The Underwater Light Field") constitutes such a superb manual for would-be modellers that it may well persuade many aquatic biologists that, given the availability of modern underwater irradiance meters and microcomputers, it is very much easier to measure light than to measure photosynthesis. Part II ("Photosynthesis in the Aquatic Environment"), on the other hand, creates the impression that biologists are still far from the position where they can make much use of the exact information about underwater light described in Part I. There is, therefore, little incentive for physicists to become involved with photosynthetic problems and to reciprocate the movement of biologists into their field. This is a familiar problem at the boundaries of biology and physics, but one which is accentuated to a possibly unnecessary degree by the contrast between the two parts of Dr Kirk's book.

Part I is rigorous, lucid and concise. A review of the optical properties of water leads into thorough accounts of what to measure and how to measure it, and how these measurements can be used to characterize fully the underwater light field. Biologists may wonder from time to time if they really need to know all of the details discussed, but Dr Kirk provides clear, complete answers to their commonest questions, together with valuable compilations of measurements from water bodies around the world. Here, at last, is the complete manual on underwater optics for biologists, which must become an essential reference for students of all photobiological processes underwater.

The excellence of Part I sets such a high standard that it may seem churlish to complain that Part II fails to live up to it. The problem lies partly in the nature of the subject matter — the physiology and

ecology of diverse and variable organisms cannot be treated as rigorously as the physical properties of light and water — but also in the author's approach. Acknowledging that light is not the only controlling factor, he has attempted a reasonably comprehensive account of aquatic photosynthesis, in which few references to underwater optics are couched in more than general and qualitative terms. Consequently, those readers who know enough about aquatic photosynthesis to appreciate and benefit from Part I are liable to be disappointed by Part II. Instead of retracing the steps of other recent books and reviews, I feel that Dr Kirk should have tried to illustrate how our understanding of aquatic photosynthesis might be advanced by applying the knowledge of underwater light presented in Part I. Bridge builders must establish their bridgeheads before advancing into the hinterland. □

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Seeing new light

Alan E. Shapiro

Optics after Newton: Theories of Light in Britain and Ireland, 1704–1840.

By Geoffrey Cantor.

Manchester University Press: 1984.

Pp.257. £20, \$25.

THE traditional history of optics from the publication of Newton's *Opticks* in 1704 to 1840 runs roughly as follows. The Newtonian emission or projectile theory of light dominated the wave theory in the eighteenth century, which, however, was overall a "sterile" period for optical research. The wave theory began to gain the upper hand at the beginning of the nineteenth century, when Thomas Young discovered the principle of interference, although initially his work was unjustifiably ignored. Finally, within a decade of Augustin Fresnel's comprehensive formulation of the wave theory (1815–1825), that theory vanquished its rival.

Geoffrey Cantor's primary aim in *Optics after Newton* is to reinterpret many elements of this story. He rejects the "sterility" thesis for the eighteenth century; he adds a third major theory of light, fluid theories, in which light is conceived to be a subtle fluid of interacting particles — not independent projectiles — flowing from a luminous source; and he argues that Young's principle of interference was rejected because it was perceived to be part of his otherwise unoriginal wave theory of light. I find Cantor's reinterpretation to be largely unconvincing. The primary cause of this is that the author is more concerned with historiography than with the history of optics, which he

uses to test various ideas about scientific communities, natural philosophy and scientific change.

Cantor takes a sociological approach. This is most fruitful in the treatment of the surprisingly large number of fluid theorists that he has uncovered, for they were for the most part attempting to use theories of light in Biblical interpretation and were not engaged in science, by making predictions, doing experiments or explicating optical phenomena. By Cantor's own account, they were marginal to the British scientific community at the beginning of his period of study and irrelevant by its end.

When Cantor turns to more serious scientific theories, such as the Newtonian emission theory, his sociological concepts are too narrowly conceived. For instance, he argues that no research occurred in the emission theory from 1700 to 1740, because its proponents were diverted by teaching. Besides the simple confusion of a cause and a correlation, this sort of claim could readily be tested by a comparison with the situation on the Continent during that period. Indeed, Cantor's self-imposed restriction to Britain and Ireland is a serious flaw, for a strong case can be made that the British Isles were a scientific backwater for most of the period from 1704 to 1840, so that what he has given us is actually an account of a relatively stagnant scientific community, rather than a progressive one. Thus, at the beginning of the nineteenth century, when he declares the emission theory to have been in decay in England, it was flourishing in France in the hands of Laplace, Biot and Malus.

An explanation for the initial neglect of Young's contributions — the most important and original British work in this study — provides, as Cantor recognizes, a critical test of his account. I am, however, only partly persuaded by his solution, namely, that the principle of interference was rejected because it was associated with Young's unoriginal wave theory. Cantor underestimates the quality of Young's work and depends too much on denigrating both him — Euler's "English disciple of moderate competence" — and his work, which he does not fully and clearly explain. He also introduces an artificial distinction that too sharply differentiates Young's and all earlier "vibration" theories from Fresnel's mathematical "wave" theory, whereas I believe there is a genuine continuity from Huygens through Euler and Young to Fresnel.

The most valuable feature of *Optics after Newton* is the vast quantity of primary literature described by Cantor, which can serve as a base for future work on the history of optics, not just British theories of light. □

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Firsts for feminism

Morris Kline

A Convergence of Lives: Sofia Kovalevskaja, Scientist, Writer, Revolutionary.

By Ann Hibner Koblitz.

Birkhäuser: 1984. Pp.305. \$19.95.

WHY should anyone be interested in a biography of someone whose name, much less her work, is known to very few people and who died nearly one hundred years ago? The chief reason for this fine biography is that Sofia Kovalevskaja was a first-class mathematician, who achieved a place for herself in a period when women were hardly recognized outside the home.

In her time, Kovalevskaja achieved high professional status; she was the first woman to be awarded a doctorate in mathematics, the first (outside Italy) to become a professor of mathematics, and the first to serve as editor of a major mathematical journal. She also made impressive contributions to literature, and engaged in social and political efforts in the cause of women's rights. All of these activities were carried on during a life span of only 41 years (1850-1891) and despite the inevitable opposition from male professors in mathematics and other fields.

Kovalevskaja had become attracted to mathematics even as a child, when she was instructed by tutors. To counter the opposition to women as students in the higher Russian academic institutions, she managed by a rather frequently used subterfuge, called fictitious marriage — legal but not sexual — to get out of the country in order to study mathematics. Most of the book is devoted to her development, to the lives of her immediate family and friends, and to her troublesome relations with her husband, who was a geologist. Later, she and her "fictitious" husband did become sexually involved; the marriage produced a daughter.

Kovalevskaja's pursuit of mathematics was her main drive in life. After leaving Russia, she became a student at the University of Heidelberg where she spent several years. There she learned of the work of Karl Theodore Weierstrass (1815-1897),

one of the leading mathematicians of Europe and a professor at the University of Berlin. Though she was denied admission to the university, Weierstrass was greatly impressed with her ability and took her on as a private student. With his backing she received a doctorate in mathematics.

Fortunately, Gösta Mittag-Leffler, a liberal Swedish mathematician, then sponsored her for a professorship at the University of Stockholm, a post she took on leaving Berlin and held for many years. During these years, and even earlier, she came to know the leading mathematicians of Europe; it was through recognition in this way, and despite considerable opposition from the establishment, that she was elected to the Russian Imperial Academy of Sciences.

To those who are primarily concerned with the history of mathematics as such, this book will be a little disappointing. There is little mention of Kovalevskaja's actual work, beyond a list of her publications. But it is clear that whereas some mathematicians make brilliant creations as youths, Kovalevskaja developed slowly. In her case the educational difficulties she faced were at least partly responsible, and had she lived longer she would have surely produced much more. Equally interesting to historians of the intellectual connections in the nineteenth century are many of the remarks and details concerning the mathematicians with whom Kovalevskaja kept contact. In particular, her relationship with Weierstrass, who practically adopted her as a daughter, reveals much of that man's noble character.

Ann Hibner Koblitz quite clearly has done a great deal of research in Russia and Stockholm to write this lucid biography. The bibliography is immense and will be of great value to other scholars. Her book should also be of interest to a broad audience, as an example of what a woman could accomplish in nineteenth-century anti-feminist Russia and for the insight into the political and social climate of that period. My only quibble is the word "revolutionary" in the title. Kovalevskaja was indeed what we nowadays know as a feminist. But she was not a militant. □

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Science and Civilization in China Vol. VI Part II *Agriculture* by Francesca Bray has recently been published by Cambridge University Press and will be reviewed in a forthcoming issue of *Nature*.

First steps in spectroscopy

K. A. McLauchlan

Interpretation of NMR Spectra: An Introductory Audiovisual Programme.

By J.D. Coyle, E.J. Haws, K. Miller and K. Norton.

Wiley: 1984. £199.

WILEY's new audiovisual programme comes in a striking package, a large boxed folder which contains four tape cassettes and four boxes (in which are a total of 164 coloured slides), and also 20 booklets. Each tape deals with a particular subject — the chemical shifts of protons, the spin-spin coupling of protons, use of proton spectra for structural assignment and all three of these topics applied to ^{13}C spectra — and is complemented by the slides. Run freely each tape lasts for about 40 minutes, although the package is designed to be interactive with the student so that he may stop and start the tape at will so as to suit his own pace of learning. This is best accomplished with a dedicated slide-tape machine, but an ordinary tape recorder and a slide projector can be used if necessary.

Two types of booklet are supplied, ten of each, together with a correlation chart of proton chemical shifts and a listing of proton coupling constants. The latter is inadequate, even at this level, as is the correlation chart for ^{13}C shifts which is found inconveniently on a slide. One booklet is a very simplistic introduction to NMR spectroscopy. The other is a workbook which has well-defined learning objectives, and in which the student is encouraged to write his replies to answers posed on the tapes and the slides. Answers are supplied on the succeeding slides.

The course has been put together with a great deal of care; the presentation on the tapes is clear and slides are beautifully presented. The coordination of the material between booklet, slide and tape is excellent, too — in theory; my programme did not run properly but that could be a quirk of the review package. The tape cues in the operation of the slide-tape machine and the mechanism changes the slide one is looking at to correspond to what is being said, while the tape stops at pre-ordained places to allow the student to think before he goes on.

The material itself is extremely elementary, aimed primarily at trainee technicians but also perhaps at students at the school-university interface or at first-year undergraduates in organic chemistry. In its determination to be straightforward — it is after all an introductory course — it contains slight inexactitudes and half-truths which might worry the better student. Worse, however, is that the package represents the state of knowledge of the subject as it was about 20 years ago.

At this level it forms a very basic introduction to the subject which might well build confidence in the student, but if one is going further the background needed would have to be more thorough — Fourier transformation, double resonance, 2D spectroscopy and the various pulse sequences all lie beyond the scope of the Wiley course.

It is initially attractive to think of replacing myself in all those tutorials each week with a magic box, but what are its disadvantages? The publisher extols the virtue of being able to hear the commentary and see the slides at the same time, rather than have your eye skip rapidly from text to diagrams as in a book — fine, provided everyone has a slide-tape machine in his own room! More importantly, this form of teaching seems to be based upon the precept that a student learns in the classroom, but one of the aims of education

should be to free him from this restriction. A student should be inquisitive, but how is this virtue encouraged by sitting before a machine which cannot answer questions and which has been programmed by someone else's narrow concept of what should be taught?

Nuclear magnetic resonance is, at best, a subtle, exciting but challenging subject which repays real effort in learning. I am afraid that there is no substitute for a live tutor willing and able to discuss its depths and its applications, to maintain this interest and sense of involvement with developing science on many frontiers. For myself, the Wiley package offers no respite from tutorials, but teachers of the subject at technical colleges may well find it a helpful adjunct to their lectures. □

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Tigers and calotypes

Brian Coe

Beyond Vision. By Jon Darius.

Oxford University Press: 1984. Pp.224. £15.

IN RECENT years there has been something of a glut of books of historic photographs. As a rule, either the photographs have been chosen to illustrate the history and development of the photographic process, or, based upon the dubious proposition that photography is Art, pictures have been selected for their supposed aesthetic merits. Very few photographs have been presented for their scientific importance — yet, as Dr Darius makes clear in his introductory essay, from its inception photography has been employed to great purpose by scientists. The collection of 100 historic scientific photographs, well reproduced in *Beyond Vision*, is therefore all the more welcome.

Dr Darius has applied very rigid criteria in selecting the photographs, the most important being that each should be unique and should show only what cannot be seen by the unaided human eye. Since the book is presumably intended for a lay readership, it might be argued that slightly less rigid demarcations might have provided greater variety; well over 60 per cent of the illustrations are of astronomical subjects or specialized aspects of physics. In any case, some of the subjects shown appear to fall outside the limits set. The record of the Tasmanian tiger, now extinct, is still no more than a fascinating document of a subject visible to the unaided eye. And the rather pretty colour photograph of the H-bomb test at Eniwetok is no more than a plain record; why were the extraordinary sequence photographs of the first micro-seconds of the fireball made by Dr Edger-ton's highspeed camera not chosen?

The inclusion of the contentious Skaife "instantaneous" photograph of a mortar shell in flight is also a little odd. The picture has clearly been retouched, and even if it is authentic its purpose was not scientific but promotional. There are questionable omissions, too. For instance, no example of the forensic use of ultra-violet and infra-red photography is included, and there is not a single scanning electron micrograph of any sort, despite the exceedingly wide range of applications in science and industry. To be fair, in reducing the entire output of 140 years of scientific photography to 100 examples, the author could not expect to please everyone.

The introductory essay is lucid and informative but is marred by several errors. The skeleton outline of photographic processes is misleading; it makes no mention of the key fact that the calotype process made paper negatives which could be printed. To use the description "coated with emulsion" to cover such diverse processes as the Daguerreotype (a silver surface sensitized by iodine vapour), the calotype (paper impregnated with silver salts), the collodion process (a glass plate coated with halide-containing collodion and sensitized in silver nitrate) and the dryplate process (a suspension of silver salts in melted gelatin coated onto glass or paper) is carrying simplification too far. Talbot's early photomicrographs, c. 1837, were not calotypes, but were made by his earlier "photogenic drawing" process.

Seen against Dr Darius's achievement in bringing these photographs together, these are minor criticisms. Overall, the book is a most valuable contribution to the literature on the history of photography. We must hope that it will be sufficiently successful to be followed by a second selection revealing more of the wonders of scientific photography. □

Brian Coe is Curator of the Kodak Museum at Harrow, Middlesex.

New for tissue culture

The eleven items here cover recently announced products of use in the tissue culture laboratory

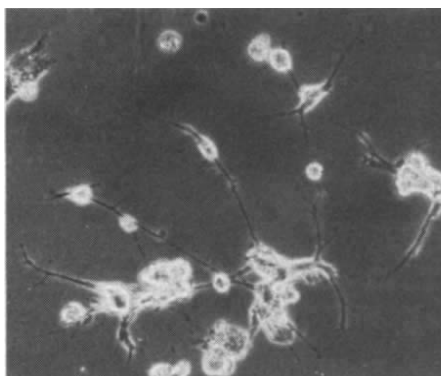
● A new range of culture dishes is now available from International Bio-Technologies (IBT) Ltd. The dishes are coated with a naturally produced extracellular matrix (ECM), a basement membrane-like substratum which promotes cell attachment, proliferation and differentiation. The ECM is secreted by endothelial cells cultured on conventional tissue culture plastic. The cells are then removed, leaving the underlying ECM intact and firmly attached to the surface of the dish. The composition and supramolecular arrangement of the ECM is similar to basement membranes occurring *in vivo*. Cells placed in contact with ECM attach rapidly, exhibit high plating and cloning efficiencies, proliferate rapidly, reach a high saturation density and have much lower requirements for serum and added growth factors than cells in conventional dishes. They also respond better to physiologically occurring hormones and express differentiated functions. These effects cannot be obtained by cell plating on isolated fibronectin, laminin or each of the collagen types and on reconstituted matrices. Among the cell types showing a favourable response to ECM are normal and carcinomatous human epithelial cells derived from surgical biopsies, human amniotic fluid cells, bovine granulosa, adrenal cortex and endothelial cells, rat pituitary, oligodendrocytes and dorsal root ganglia cells. The coating is available on any size of tissue culture dish, T-flasks, multiwell plates and coverslips.

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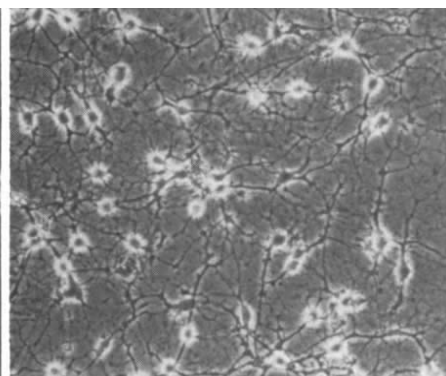
● The culture of hybridomas and diploid human cells has become increasingly important and this has led LSL Biolafitte of Princeton, New Jersey, to design a range of vessels specifically suited to this purpose. LSL Biolafitte offers eight vessels with working volumes from one litre to 330 litres. Each vessel has optimized geometry with a working height to diameter ratio of 1:1 and hemispherically shaped base. An effective low speed, low shear agitation system is incorporated into each vessel to gently agitate the cells. Harvesting is achieved by a conveniently located valve which has two positions allowing total drainage or liquid removal only. Other system design features are included, or are available as optional accessories, to ensure strict containment in processes where this is an important factor.

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These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Service Card bound inside the journal.



Adult rat oligodendrocytes maintained on conventional plastic (left) and on plastic coated with IBT's extracellular matrix preparation.



● The Sterilin range of tissue culture plastics includes dishes, tubes, flasks, plates and bulk vessels designed to ensure efficient attachment and growth of cell monolayers. All equipment is manufactured from non-toxic polystyrene, providing a high standard of finish and good optical properties for microscopy. Every batch is tested for sterility and the ability to support cell growth. Petri dishes are available in a wide range of sizes and types — the 50mm and 60mm dishes can be supplied with single or triple vents and a multi-well dish is also available. Sterilin test tubes come in two sizes: the 13ml tube is fitted with a leak-proof screw cap, and the 5ml tube has a two-position cap for gas exchange. For research and clinical studies involving human and animal cells, two sizes of tissue culture flask are available. Graduated in mls, the wide necks (canted in the 25cm² size) allow easy pipette access and non-toxic leakproof screw caps are fitted for safety. A stacking rim is provided for extra stability. All flasks are individually pressure-tested to eliminate the possibility of leakage through the welded seams.

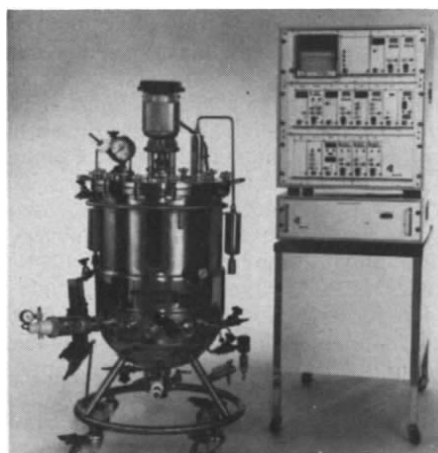
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● Sera-Lab fetal calf sera are now available in graded batches to select those which will support the rat and mouse myelomas used to make cell hybrids. Each batch of serum is routinely tested for the ability to support the growth of 11 cell lines, so at no extra cost the customer can request a serum to suit a particular need.

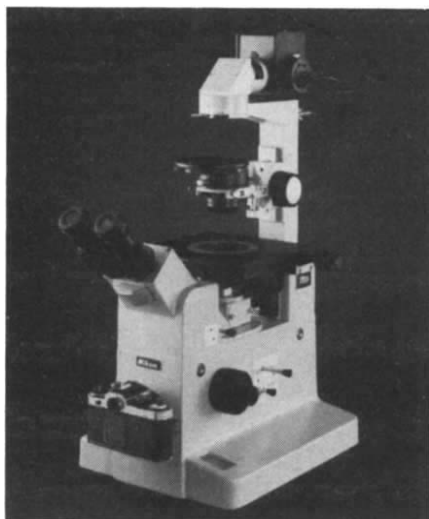
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● A kit for preparing serum-free media has been announced by Gibco. The Select-a-Factor kit contains 1 ml sample sizes of the more commonly-required factors and technical information on how to grow cells in a serum-free environment. The technical information describes four experiments that explain the preparation of serum-free media. The kit includes 1 litre of powdered medium (Dulbecco's minimum essential medium: nutrient mixture F-12 with 15-mM HEPES), trypsin inhibitor and 14 ready-to-use factors (EGF, FGF, fibronectin, poly-D-lysine, vitamins C and E, insulin, transferrin, hydrocortisone, progesterone, selenium, putrescine, trace element mix, and T₃). The factors are also available individually for the preparation of subsequent media.

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One of the LSL Biolafitte culture vessels (left) and the Sterilin range of tissue culture plastics.



The Nikon Diaphot tissue culture microscope.



Disposable filter capsules from Gelman.

● The range of accessories available for the Nikon-Diaphot inverted tissue culture microscope now includes systems for time-lapse cine photomicrography or closed circuit television that can be fitted without disturbing the camera mounted on the front. Motor driven computerized stepping stages can be fitted to the Diaphot for image analysis work, and for environmental control, the Nikon constant temperature incubator can provide warm air at 37°C. An override allows variation in temperature of plus or minus 4°C.

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Liposomes

A new laboratory instrument (LIPOPREP®-GD-1) is available for the reproducible preparation of extremely homogeneous, unilamellar liposomes of controllable size. The instrument permits the simultaneous use of five different lipid mixtures and provides extensive versatility. Applications range from membrane reconstitution, remodelling of cellular functions, liposome drug delivery systems, microanalysis of drugs and metabolites, to antibody targeting, clinical diagnostic assays, and genetic engineering.

For details write to:
DIANORM-Geräte, P.O. Box 126, D-8 München 65, FRG.

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● Gelibead, a gelatin microcarrier developed by KC Biological, solves two of the major problems associated with microcarrier culture. First, enzymatic removal of viable cells from the microcarrier is possible and second, eluted cells can be readily removed from the spent microcarriers. Gelibead microcarriers permit highly efficient cell harvesting because they completely dissolve in a mixture of trypsin, dispase and EDTA. Exposing Gelibeads for about 20 minutes to this mixture results in complete dissolution of the microcarriers with very high cell recoveries. Gelibeads can be attached to a wide range of cell types, provides high density culture (about 3,800 cm² per gram dry weight) and gives high cell yields.

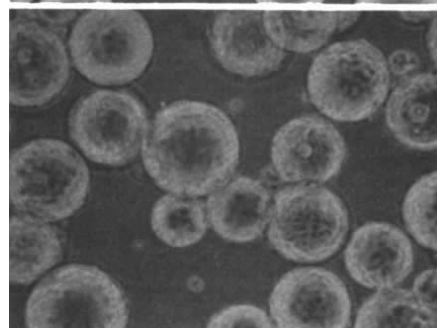
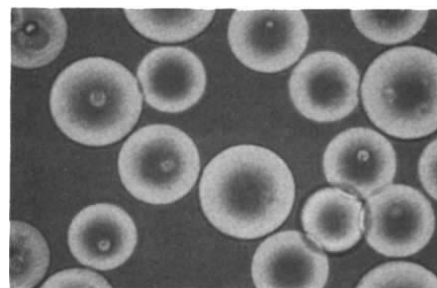
Circle No. 106 on Reader Service Card.

● In the new Luckham RMV tissue culture roller apparatus the rollers rotate at a set speed with the minimum of variation, resulting in steady even culture growth. All rollers are driven, they are covered in rubber and support the bottle along its whole length. The unit is available in two speed ranges, 2–10.5 revolutions per hour or 40–180 per hour (other speed ranges are available to special order). There is an analogue speed meter so that experimental conditions can be precisely reproduced. Luckham has also just introduced the TC 1000, a drum roller designed for the rotation of 16-mm diameter tubes. The unit has a variable speed between 0.5 and 60 r.p.m. Speed is electronically controlled, and read out via an analogue meter. Luckham manufactures a range of racks designed for tissue culture work, with a variable angle between 0 and 5 degrees.

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● Gelman's new "Culture Capsule" and "Maxi Culture Capsule" are disposable, ready-to-use capsule filtration devices for sterilizing large volumes of tissue culture media. These presterilized capsules incorporate two layers of 0.2 µm low-protein-binding HT Tuffryn membrane, and are designed and certified to assure their integrity for tissue culture media sterilization. Designed for use with positive pressure filtration, the Culture Capsule and Maxi Culture Capsule are suitable for in-line applications using a pressure vessel or peristaltic pump. For sterilization of culture media containing serum, either capsule may be used as the final sterilizing unit in conjunction with prefilters. Disposable filters minimize set-up time and risk of cross-contamination. The Culture Capsule (product no. 12140) provides 500 cm² typical filter area, suitable for sterilization of up to 10 litres of solution. The Maxi Culture Capsule (no. 12141) offers 1,290 cm² typical filter area, and is ideal for larger volume applications of up to 20 litres. Both capsules are available from the following laboratory supply dealers: American Scientific Products, Fisher Scientific, VWR Scientific and Curtin Matheson Scientific.

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The Gelibead microcarrier without cells (top) and with attached MDCK cells.



● A new product from Hana Biologics is intended to reduce the requirement for serum in mammalian cell culture. SerXtend is a combination of growth factors which when added to fetal bovine or other sera will allow for a 50% or greater reduction in the concentration of serum used in cell culture. It is a non-serum based product, formulated and certified by biological activity to allow for lot-to-lot consistency.

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● The new Microtec Swift tissue culture microscope is an inverted microscope which can be fitted with bright field or phase contrast objectives designed to work through the chambers used in tissue culture. Illumination is provided by a 20 watt halogen source and, with the standard condenser, chambers up to 45mm thick can be used. An extra long working distance condenser system, with a working distance of 60mm, is available for use with thicker chambers. A camera port is a standard feature enabling a 35mm camera to be plugged into the side of the microscope. The coaxial coarse and fine focusing mechanism operates entirely on the objectives, so that viewing head, camera and specimen stage are in a fixed position.

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